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Research Article

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Choline Pathways during Normal and Stimulated Renal Growth in Rats

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ABSTRACT Cellular membrane synthesis occurs during normal and stimulated renal growth. Choline in the kidney is utilized as a precursor for membrane synthesis via the choline kinase reaction. We investigated choline phosphorylation during normal and stimulated renal growth. Rapidly growing neonatal rat kidneys contained relatively high levels of choline kinase activity (61 pmol phosphorylcholine/min per mg protein). Choline kinase activity and phosphorylcholine production then fell gradually over the 1st mo of life; by 1 mo phosphorylcholine production was 34 pmol phosphorylcholine/min per mg protein. Choline kinase activity increased by 27% ($P < 0.001$) in 28-day-old rats when renal growth was stimulated by contralateral nephrectomy; the increase occurred within 2 h after surgery. Thus, changes in the activity of this important enzyme in the initiation of membrane synthesis is associated both with normal renal development and with adaptation to nephron loss. The findings further suggest that the cell membrane may be involved in the initiation of compensatory renal growth.

INTRODUCTION

Normal and compensatory growth in the kidney relies on the formation of new cellular membranes. Choline is utilized as a precursor for phospholipids in cellular membrane synthesis (1, 2). It is phosphorylated to phosphorylcholine via choline kinase (E.C.2.7.1.32) (ATP:choline phosphotransferase) and then synthesized into membrane phospholipids via the Kennedy pathway (2). Thus, choline kinase is an important enzyme in the initiation of membrane synthesis in this pathway. In addition, choline can also be oxidized in the kidney as a donor of methyl groups for metabolic reactions (3, 4).

We investigated the formation of phosphorylcholine from choline during normal and stimulated renal growth. Our results indicate that rapidly growing fetal rat kidneys contain relatively high levels of choline kinase activity. Choline kinase activity then decreases as choline oxidation increases over the 1st mo of life as the kidneys mature. Choline kinase activity is increased when renal growth is stimulated by acute reduction in renal mass.

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METHODS

Fetal (18–19 days gestation) and young (2–65 days) Sprague-Dawley rats (Charles River Breeding Laboratories, Inc., Wilmington, Mass.) were fed either maternally or with Purina rat chow (Ralston Purina Co., St. Louis, Mo.) after weaning. At the time of study, the rats were decapitated and the kidneys immediately removed for analysis. In some studies, 2- to 40-day-old rats underwent uninephrectomy or sham uninephrectomy of the left kidney under ether anesthesia. 2, 24, or 48 h later the rats were decapitated and the right kidneys removed.

After removal, the kidneys were immediately homogenized in four times their volume (by weight) of cold 0.05-M Tris buffer, pH 8.0, that contained 5 mM EDTA and 10 mM dithiothreitol. 40 μl of the homogenate mixture was added to a reaction mixture of final composition: 33 mM Tris buffer (pH 9.0), 11 mM $MgCl_2$, 1 mM EDTA, 1 mM dithiothreitol, 15 mM ATP, and 2 μCi of [methyl-^{14}C]-choline chloride (sp act 56.85 mCi/mmol) (New England Nuclear, Boston, Mass.) for a final choline concentration of 0.143 mM, unless otherwise stated, in a final volume of 300 μl. (V_{max} in this assay system is 41 pmol/min per mg protein.) The final pH of the mixture was 7, the optimum pH for choline kinase activity in the assay system. After incubation for 30 min at 37°C, the reaction was stopped and phosphorylcholine and betaine formation were measured by a modification of the method of Bandelin and Pankratz (5). 50 μl of a 40-mM solution of choline chloride and 550 μl of a saturated reineckate solution (Sigma Chemical Co., St. Louis, Mo.) were added to stop the reaction. (The two reaction products, phosphorylcholine and betaine, are soluble in the reineckate solution, while choline, water-insoluble products, and protein are precipitated.) The mixture stood at 4°C for 15 min, then was centrifuged for 10 min at 1,000 *g*. 300 μl of the supernate was then re-extracted with 130 μl of saturated reineckate salt solution. After re-extraction, 10 μl

of the supernate was dissolved in 10 ml of Aquasol (New England Nuclear), stored in darkness for 18 h, then counted in a Beckman LS-250 scintillation spectrometer (Beckman Instruments, Inc., Fullerton, Calif.) to a 2% error. The results were corrected for quenching. Less than 2% of the initial choline in the incubation mixture was converted to phosphorylcholine or betaine in the reineckate-soluble fraction. Under 0.2% of the choline remained unprecipitated in the supernate.

Phosphorylcholine and betaine in the supernate were separated and identified by paper chromatography. 6 μl of the supernate was applied to Whatman 3M paper strips (Whatman, Inc., Clifton, N. J.) and separated by ascending chromatography in a solvent system of butanol:acetic acid: water (5:2:3), or propanol:80% formic acid:water (7:2:1) at room temperature. The strips were cut into 0.5-cm pieces, shaken in Aquasol, and counted. Only two peaks were found in the reineckate fraction; these were identical in both solvent systems to standards of [methyl-^{14}C]phosphorylcholine and [methyl-^{14}C]betaine (New England Nuclear). Virtually all of the radioactivity of the standards applied to the paper was recovered in the peaks.

The percentage of phosphorylcholine or betaine in the reaction was calculated from the respective areas of the chromatographic peaks × 100, divided by the sum of these areas. The amount of radiolabeled phosphorylcholine or betaine was calculated by measuring the total radioactivity in a 10-μl sample and multiplying by the percentage of phosphorylcholine or betaine in the sample.

Choline kinase activity was estimated as counts per minute phosphorylcholine formed per minute per milligram protein. Choline oxidation was estimated as counts per minute betaine formed per minute per milligram protein. The data were then converted to picomoles choline incorporated into either product.

Protein determinations on the extract were done by the Lowry method (6).

RESULTS

The formation of phosphorylcholine and betaine was a linear function of time over the 30-min incubation period. The measured K_m for choline kinase activity was 25 μM and the V_{max} was 141 pmol/min per mg protein. An Augustinsson plot of the data for choline oxidation revealed two slopes. The K_m for the lesser slope was 3.8 mM. The V_{max} for the choline oxidase system was not calculable, from 100 μM to 5 mM.

Normal development. 50 rats were studied during normal development. In the renal homogenates the sum of radioactive choline incorporated into phosphorylcholine plus betaine remained generally constant at 74±5 (SE) pmol choline/min per mg protein, throughout the 1st mo of life. In kidney homogenates from fetal rats of 18–19 days gestation, the activity of choline kinase was 153±28 pmol/min per mg protein. No betaine was formed. By 2 days of age, choline oxidation was evident (Fig. 1). A linear decrease in phosphorylcholine formation ($r = 0.97$) occurred during development, while betaine formation increased during this period. By 14 days the ratio of phosphorylcholine to betaine formation was 4:1; by 1 mo, the ratio was about 1:1, and by 2 mo the ratio fell further, to 2:3.

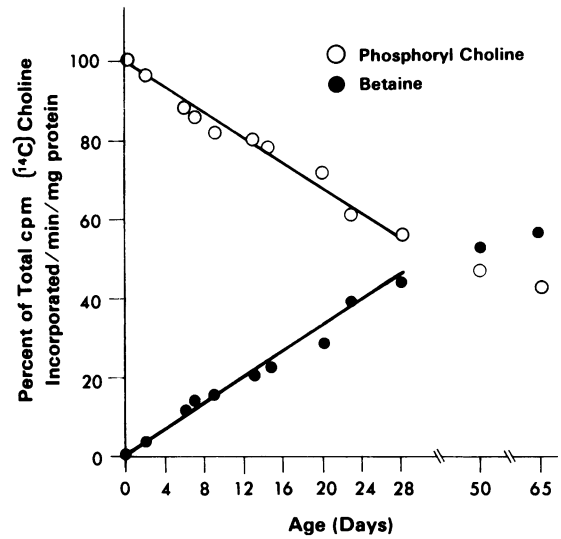

FIGURE 1 Effect of age on phosphorylcholine formation and oxidation during development. Renal homogenates of young rats were incubated for 30 min with [^{14}C]choline (see text). The amount of [^{14}C]phosphorylcholine formed is a measure of choline kinase activity; the amount of [^{14}C]betaine formed is a measure of choline oxidation. Each value represents the mean of two–four animals. Ordinate: mean value of each product formed ×100/sum of mean values of both products; abscissa: age of animal, in days.

Renal compensatory growth. The effects of uninephrectomy on choline kinase activity varied with the age of the rat. Until 14 days of age, rats that underwent uninephrectomy showed a 14% increase in choline kinase activity in the remaining kidney, compared to sham-operated animals ($P < 0.01$) (Table I). At 21 and 28 days, the activity of choline kinase in the remaining kidney was also significantly increased in the remaining kidney compared to normal.

After 2, 24, or 48 h of renal compensation in 28- to

TABLE I
Renal Choline Phosphorylation after 24 h of Renal Compensatory Growth

Rat age		Phosphorylcholine			UNx
		UNx	Sham	*P* value	Sham
days	*n*	*pmol/min/mg protein*			
2–7	9*	69.3±3.5	60.9±2.6	<0.01	1.14
14	8‡	47.6±2.5	48.2±2.4	NS	0.99
21	6‡	46.9±4.9	40.1±5.4	<0.01	1.17
28	7	42.9±4.2	33.8±4.5	<0.001	1.27

Renal homogenates from uninephrectomized (UNx) and sham-operated (sham) rats were incubated with [^{14}C]choline and the amount of [^{14}C]phosphorylcholine formed was determined. Results are means±SE.

* Pool of three–four kidneys for each experimental value of *n*.

‡ Pool of two kidneys for each experimental value of *n*.

40-day-old rats, an average increase in choline kinase activity of 23% over control values was found in the compensating kidney (Table II). Choline oxidation was unchanged during renal compensation.

DISCUSSION

Our results indicate that the entry of choline into the pathway for membrane synthesis varies with the degree of renal growth. The fetal rat kidney contained high levels of choline kinase. The level then gradually declined during the 1st mo of life. Choline oxidation, as measured by betaine formation, was not detectable in the fetal kidneys at the medium concentration of choline tested. 2 days after birth, betaine formation was measurable; it increased linearly as phosphorylcholine formation declined, until 1 mo of age, when the activities of both enzyme systems were similar. During stimulation of renal growth by contralateral nephrectomy, between 1 and 2 mo of age, choline kinase activity increased whereas choline oxidation changed little or not at all.

These findings further extend our previous reports that choline incorporation into phosphatidylcholine is increased during this period in response to acute nephron loss in the rat and mouse kidney (7) and in response to renal ischemic injury (8). The rapid response suggests that the cell membrane may be involved in the early regulation of cell growth (9–11) and may initiate the response to the loss of nephrons (7).

The increase in enzyme activity may be the result of activation of existing enzyme or the formation of new enzyme, since new cellular proteins are formed by 14 h after the onset of renal compensatory growth (12). The newly synthesized phosphorylcholine is needed not only for the membranes of the new daughter cells after mitosis, but for increased amounts of Golgi membranes and microtubules (13) and an increase in the number of mitochondria (14). The pathway is probably a major cause for the 33% increase in phospholipid in the compensating kidney that occurs by the 6th day of growth (15).

TABLE II
Renal Choline Phosphorylation in Young Rats after Uninephrectomy

Hours of renal compensatory growth		Phosphorylcholine			UNx
		UNx	Sham	*P* value	Sham
h	*n*	*pmol/min/mg protein*			
1–2	10	49.5±3.1	41.8±2.0	<0.025	1.18
24	9	40.8±3.5	32.2±3.6	<0.001	1.27
48	7	51.5±3.7	41.2±1.1	<0.05	1.25

Rats underwent uninephrectomy (UNx) or sham-uninephrectomy (sham). At various hours after surgery, their kidneys were removed, homogenized, and incubated with [^{14}C]choline for 30 min. The amount of [^{14}C]phosphorylcholine formed was a measure of choline kinase activity. Results are means±SE. Paired *t* tests were used for an analysis. Age of rats, 28–45 days.

The sum of choline kinase and choline oxidase activities in the present study was 74 pmol choline incorporated into product/min per mg protein. This value is similar to the value of 50 pmol that can be calculated from data utilizing renal slices or homogenates from rats of unspecified age and weight (16). From those data, the choline kinase activity can be calculated as only 14% of the activity of choline oxidation in slices and under 1% of the activity of choline oxidation in homogenates. The reason for the different values for the slices and homogenates is not apparent.

The concentration of choline in our incubation media (143 μM) saturated choline kinase at pH 7.0 and 37°C. Under these conditions our measured K_m was 25 μM and V_{max} was 141 pmol/min per mg protein. Haubrich (17) showed that partially purified choline kinase from the rabbit brain is saturated at low concentrations of choline (40 μM at physiological pH; K_m was about 30 μM.)

An Augustinsson plot of the data for oxidation revealed two slopes. The K_m for the lesser slope was 3.8 mM, which is close to that found by DeRidder et al. (18) in the rat liver (1.2 mM). The V_{max} for the choline oxidase system could not be measured by our assay within a wide range of [^{14}C]choline concentrations. It has apparently not been reported elsewhere. This system may be unsaturated in vivo, and may be similar to another choline enzymatic reaction, choline acetyltransferase, in brain, which is not saturated in vivo (19).

In general, there is a greater degree of renal compensatory growth, the younger the animals (20–25). However, in the present study, choline kinase activity increased only slightly during compensatory growth in rats younger than 14 days of age. The enzyme is probably optimally active in this early, relatively intense period of normal growth, and the rat kidney is still immature in the first 2 wk of life.

14 days of age marks a turning point for several parameters of growth in the developing rat. (*a*) Before then, hyperplasia is the dominant mechanism for renal compensatory growth. After then, both hyperplasia and hypertrophy are the major mechanisms (20, 26, 27). (*b*) At 14 days of age, the levels of RNA in the renal cortex decrease transiently with respect to DNA (20). (*c*) Cyclic AMP levels, which rise by 50% between days 4 and 7, decrease at day 14 (28). (*d*) Gross changes, as the opening of eyes and the sudden appearance of body hair, also occur on the 14th day. Thus, our observation of decreased choline kinase activity at this day, although still unexplained, is accompanied by other sudden alterations in growth factors.

The choline kinase pathway, which favors the pathway of choline to phosphatidylcholine, is relatively high in other rapidly growing or fetal tissues, e.g., fetal lungs of the rat, rabbit, and human (29–31). Choline kinase in the rat is three to four times higher in rapidly dividing tissues, e.g., the liver or brain (16). Relatively low rates of choline oxidation have also been found in mitochondria from fetal rat livers (32). The rate rises markedly as the rats mature to 20 days, similar to our findings for the kidney.

In our study the increase in choline kinase activity during renal compensatory growth began early, within 2 h of nephrectomy, and lasted over the 48-h period tested. During this time, other biochemical events of renal compensatory growth, such as protein, RNA, and DNA synthesis, are at their peaks (21, 24, 25, 15). Thus, a key metabolic pathway for membrane synthesis remains activated during the period of maximal response to the loss of nephrons.

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