

## Evidence for Altered Cyclic Nucleotide Metabolism During Compensatory Renal Hypertrophy and Neonatal Kidney Growth

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In adult male Sprague-Dawley rats contralateral nephrectomy was followed by an initial fall of the concentration of cGMP in renal cortical tissue followed by a rise to a peak level of 300 percent of the initial concentration within two hours. cGMP concentration in the remaining renal cortex remained at about 300 percent of the initial value during the subsequent 72 hours and slowly declined to 150-200 percent in the following two weeks. The changes in cGMP concentration were due to exactly parallel changes in the soluble fraction of renal cortical guanylate cyclase activity, while cGMP-phosphodiesterase activity remained unchanged. cAMP concentration after contralateral nephrectomy fell significantly by about 25 percent within two hours and remained below baseline level for up to eight hours. In the kidneys of newborn rats the concentration of cAMP was approximately one-half that found in adult kidneys: it slightly fell between the fourth and the seventh day after birth and subsequently continuously rose to reach adult values approximately two weeks after birth. The concentration of cGMP was significantly greater four days after birth than in adult rats, further rose between the fourth and the seventh day after birth and subsequently gradually declined to adult levels. The increased cGMP concentration appears to be due to an increase of guanylate cyclase activity in total kidney homogenates which, in turn, was mainly due to an increase of the particulate (membrane-bound) fraction of the enzyme. cGMP-phosphodiesterase activity, however, was also increased in respect to adult levels, one or three weeks after birth. Renal growth from the seventh day after birth to adulthood is accompanied by a continuous increase of the ratio cAMP/cGMP. Removal of one kidney four to seven days after birth resulted in a slower increase of this ratio. The data suggest that cGMP may trigger renal growth and that increases of cGMP concentration in the kidneys are the result of a primary increase in the activity of guanylate cyclase.

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There is evidence for involvement of cAMP and cGMP in the regulation of cellular growth and proliferation. In some cell culture systems, an increase in cellular cAMP content has been shown to be associated with arrest or inhibition of growth whereas increased cellular cGMP concentrations seemed to favor growth. Furthermore, exogenous cGMP could replace different growth stimuli, whereas exogenous cAMP exerted an inhibitory effect.

It has therefore been hypothesized, that cGMP may mediate the actions of mitogenic agents, whereas cyclic AMP may inhibit cell proliferation [1]. Controversial as this thesis may be a number of animal studies have recently shown changes in the cGMP system during organ development and hypertrophy.

Thus an increase in the particulate, i.e., membrane-bound guanylate cyclase, has been reported in fetal and newborn rat liver [2], and in transplantable Morris renal tumors [3], and increases in cGMP dependent protein kinase activity of fetal and neonatal lung and heart have been demonstrated in the guinea pig [4]. Furthermore an increase in particulate guanylate cyclase activity of regenerating liver has been

reported by two groups [2,5]. We therefore investigated cyclic nucleotide metabolism during the rapid growth periods of compensatory renal hypertrophy in adult rats, during normal neonatal kidney growth, and in compensatory renal growth of newborn rats [6,7,8,9].

Unilateral nephrectomy was performed on adult male Sprague-Dawley rats or on litter mates of newborn rats of different ages and the contralateral hypertrophying kidney was removed at specified time intervals. Kidneys were snap frozen and the cyclic nucleotides were extracted by homogenization in perchloric acid.

cAMP and cGMP from the supernatant were separated on a Dowex column and determined by radioimmunoassay.

Guanylate cyclase was prepared by homogenizing fresh renal tissue in Tris buffer containing sodium EDTA. In the studies on compensatory renal hypertrophy slices of outer cortices were used whereas in the newborn whole kidneys were used. Soluble and particulate enzyme fractions were separated by centrifugation at one hundred thousand times g, for 60 minutes. Guanylate cyclase activity was measured by the method of Murad [10].

A crude preparation of phosphodiesterase was obtained from the one thousand times g supernatant of renal cortical homogenate and the cGMP phosphodiesterase activity was determined by the method of Rosen using paper chromatography for separation of the reaction products [11].

The changes in cAMP content of the hypertrophying kidneys are shown in Fig. 1. Each value represents the mean  $\pm$  SEM of six animals. In control kidney cortex of adult male rats cAMP content was 1,920 picomoles per gram wet kidney weight. A significant, though moderate and short-lasting decline of tissue levels to 75 percent of control was observed at 2, 4, and 8 hours after contralateral nephrectomy and at 24 to 72 hours baseline levels were reached again, and increased to 150–200 percent at 1 to 2 weeks (not shown). Sham operated rats showed no significant differences.

In contrast to cAMP, cGMP levels underwent marked and rapid changes (Fig. 1). In control renal cortex cGMP values were 31 pmol per gram wet weight. Fifteen minutes after contralateral nephrectomy they dropped to 25 percent of control, then rapidly increased to 200 percent at 1 hour, and 300 percent at 2 hours and remained elevated up to 72 hours and are still 150 to 200 percent at 1 to 2 weeks (not shown). These changes are statistically significant. No change in cGMP content was found in sham-operated animals. The changes in cAMP and cGMP concentrations cannot be related to changes in water content since the results are the same when concentrations are expressed per mg protein.

To elucidate the mechanisms for the altered tissue content we studied the enzymes involved in generation and destruction of cGMP, i.e., guanylate cyclase and cGMP phosphodiesterase.

The activity of the cGMP phosphodiesterase did not change at any time interval (Fig. 1). Thus, the observed changes in renal cGMP levels are probably not due to altered rates of cGMP hydrolysis. The distribution of guanylate cyclase activity between the one hundred thousand times g supernatant and the pellet of renal cortical homogenates is shown in Table 1.

About 80 percent of the enzyme activity was associated with the soluble fraction and this fraction also had a 3–4-fold higher specific activity than the crude homogenate. Therefore, only the soluble enzyme fractions were assayed at different time intervals.

The pattern of the change of the soluble guanylate cyclase in hypertrophying kidneys is shown in Fig. 1.

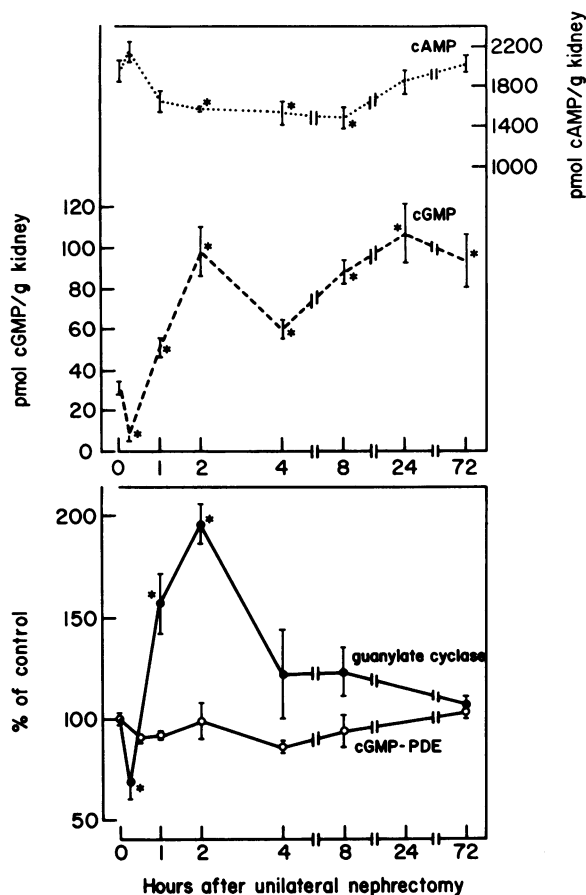


FIG. 1. The changes in renal cortical cAMP, cGMP, soluble guanylate cyclase and total cGMP-phosphodiesterase (cGMP-PDE) at various time intervals after unilateral nephrectomy. Each point is the mean  $\pm$  SEM of 6 rats for the tissue levels and of 3-4 rats for the enzyme activities. Asterisks indicate values that are significantly different from their respective controls ( $P < 0.05$ ). (From [8].)

Guanylate cyclase activity showed a significant decline to 70 percent of control at 15 minutes. One hour after unilateral nephrectomy, guanylate cyclase activity was increased to 160 percent, and at two hours it was increased to 200 percent of control and then decreased to 120 percent at 4, 8, and 72 hours. The changes at 15 minutes, 1 and 2 hours are statistically significant. Guanylate cyclase activity of sham-operated

TABLE I  
Distribution of Guanylate Cyclase Activity in Rat Renal Cortex  
 $n = 6$

Homogenate	Cyclic GMP formed (pmol/min per mg protein)	
	Soluble	Particulate
31.3 $\pm$ 3.3	118 $\pm$ 5.3	18.3 $\pm$ 1.3

All kidneys were removed from adult rats under ether anesthesia and guanylate cyclase was determined in homogenates, and in soluble and particulate fractions after centrifugation at  $100,000 \times g$  for 60 min. Values are means  $\pm$  SEM.

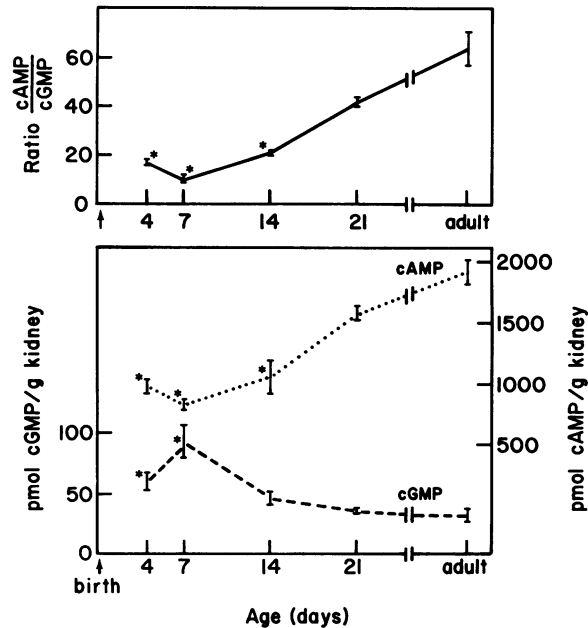


FIG. 2. Changes in renal tissue levels of cAMP and cGMP at various stages of development after birth, and in adult male rats. Each point is the mean  $\pm$  SEM of 4 newborn animals and 6 adult animals. The ratio cAMP/cGMP was calculated from the values obtained per g of wet kidney weight. Asterisks indicate values that are significantly different from the control adult animals ( $P < 0.005$ ). (From [8].)

animals was not different at any time interval. As the soluble guanylate cyclase activity showed a similar pattern as cGMP tissue levels in the initial phase, changes in cGMP synthesis could explain the altered tissue levels. Furthermore changes in the particulate guanylate cyclase at later time points cannot be excluded as we did not measure them.

We then determined cyclic nucleotide metabolism in rapidly growing kidneys of newborn rats during the first 3 weeks after birth. This period of kidney growth was associated with changes in the cyclic nucleotide systems.

Renal cAMP tissue levels at 4 and 7 days after birth were 980 pmoles cAMP per gram wet weight and 830 pmoles, respectively (Fig. 2). The level increased to 1,060 at 14 days and to 1,520 pmol at 21 days. The difference between the 4- and 7-day-old and the 21-day-old or adult animals are highly significant ( $p < 0.005$ ). Cyclic GMP concentrations underwent changes in the opposite direction. They were 60 and 92 pmol cGMP at 4 and 7 days after birth and these values are statistically different from 21-day-old and from adult animals ( $p < 0.005$ ). At 14 days the levels were 46 and at 21 days 36 pmol cGMP, which is not different from adult levels. To further elucidate the changes in cGMP levels, we measured the activities of guanylate cyclase and cGMP phosphodiesterase in the kidneys of neonate rats of different ages.

The activity of the homogenate as well as soluble and particulate enzyme is significantly higher in the newborn kidneys than in adults with the greatest increase occurring in the particulate fraction. This increase in particulate enzyme activity is still present in three-week-old animals but the activity of the soluble fraction is no longer significantly different from adults. The recovery of total enzyme activity is consistently above one hundred percent, possibly indicating removal of an inhibitor or activation of the particulate enzyme fraction during preparation.

When enzyme activity is expressed as percentage of respective adult controls the following picture emerges (Fig. 3): in the adult rat the particulate guanylate cyclase accounts for about 28 percent of the total activity, i.e., the sum of particulate and soluble enzyme fraction. In contrast the particulate enzyme of newborn kidneys is markedly elevated and amounts to 50 percent of the total activity at 1 week of age and to 40 percent at 3 weeks of age. Thus the increase in total guanylate cyclase activity occurs predominantly because of an increase in the activity of the particulate enzyme.

We then investigated phosphodiesterase activity for cGMP in these kidneys at 10 and 250  $\mu\text{M}$  substrate concentration. The enzyme activity of newborn kidneys is twice as high at 1 and 3 weeks of age compared to adults, irrespective of substrate concentration. Kinetic studies were performed and failed to show any change in substrate affinity of the phosphodiesterase as determined by the respective  $K_m$  for cGMP.

As (in contrast to the adult) compensatory renal growth in the newborn period is characterized by predominant cellular hyperplasia, cyclic nucleotide levels were determined in neonatal kidneys two weeks after unilateral nephrectomy.

When unilateral nephrectomy was performed in 4-7-day-old neonates the weight of the remaining kidney increased to 65 percent above that of normally growing control kidneys at 3 weeks of age. cAMP content in the hypertrophying kidney did not increase as in the normally growing kidney, but even showed a further decline. cGMP concentrations, on the other hand, reached the same level at three weeks with and without hypertrophy.

Thus, when the observed changes are expressed as the ratio of cAMP to cGMP concentrations a progressive increase toward adult levels is observed in the normally growing kidney. In contrast, with compensatory renal growth in the newborn the cAMP-cGMP ratio is maintained at the newborn level (Fig. 4).

In conclusion, normal neonatal growth and compensatory renal hypertrophy in the adult and newborn rat are associated with marked changes in cyclic nucleotide metabolism. These findings indicate increased activity of the whole cGMP system in

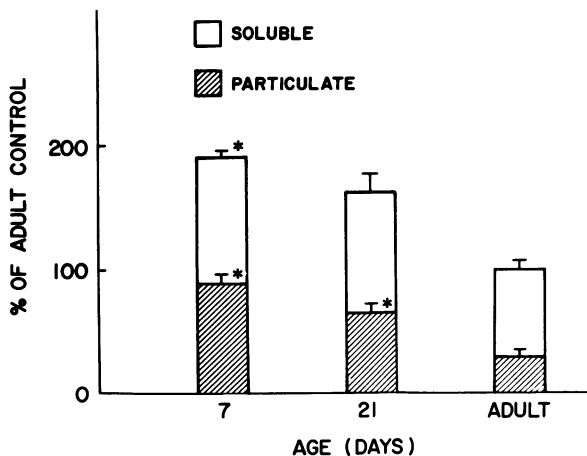


FIG. 3. Comparison of guanylate cyclase activity in the soluble (100,000 x g supernatant) and particulate (100,000 x g pellet) fraction of neonatal rat kidneys. Activities are the sum total of soluble and particulate enzyme activities, and are expressed as percentage of the respective adult control values. Each bar represents the mean  $\pm$  SEM of 4-6 animals. Asterisks indicate values that are significantly different from adult controls ( $P < 0.001$ ). (Schlondorff, unpublished observation.)

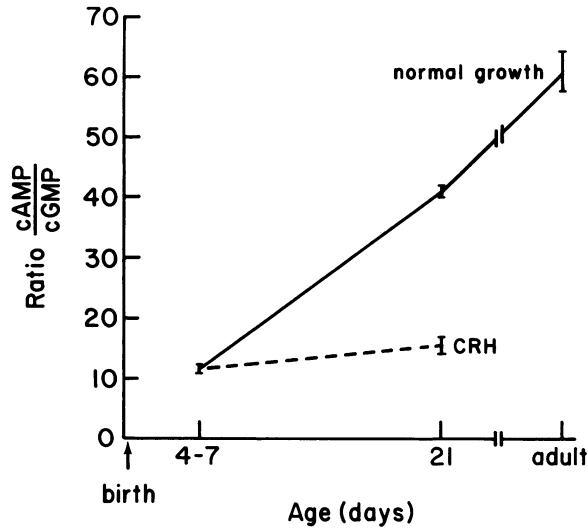


FIG. 4. Comparison of the ratio of cAMP/cGMP tissue levels during normal neonatal kidney growth and after induction of compensatory renal hyperplasia (CRH) by unilateral nephrectomy in 4-7-day-old rats. The ratio cAMP/cGMP was calculated from the values obtained per g of wet kidney weight. Each point is the mean  $\pm$  SEM of 4 newborn rats or 6 adults. (Schlondorff, unpublished observation.)

kidneys of newborn rats and fit into the pattern observed in other developing organs. These changes may not only reflect kidney growth, but may also be important factors in functional adaptation to altered homeostatic requirements.

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