Effects of Maternal Uninephrectomy on the Development of the Proximal Tubule in Fetal Rat Kidney: Morphometrical Study

Toshiya OKADA, Tatsuro YAMAGISHI, Yasuo KISO, Yoshio MORIKAWA, and Fumihiko SASAKI

Department of Veterinary Anatomy, College of Agriculture, Osaka Prefecture University, 1-1 Gakuen-cho, Sakai, Osaka 593, Japan

(Received 4 September 1995/Accepted 24 November 1995)

ABSTRACT. The kidneys of fetal rats from uninephrectomized mothers were investigated morphometrically on days 18, 20, and 22 of gestation. Maternal uninephrectomy was performed on day 5. On days 20 and 22, the proximal tubular cell height in fetuses from uninephrectomized mothers was significantly lower than that in fetuses from sham-operated mothers. The luminal diameter, length and total volume of the proximal tubule were significantly larger in fetuses from uninephrectomized mothers than in those from sham-operated mothers. The total glomerular volume of mature type in fetuses from uninephrectomized mothers was significantly larger than that in fetuses from sham-operated mothers. On day 18, maternal uninephrectomy caused no significant difference in any kind of parameters used for the proximal tubule, but induced an increase in the total glomerular volume of immature type. These results suggest that maternal uninephrectomy stimulates morphological development of the proximal tubule in the fetal kidney. — **KEY WORDS:** maternal uninephrectomy, morphometry, proximal tubule.

J. Vet. Med. Sci. 58(5): 413–417, 1996

The fact that the kidney begins to function during intrauterine life [2] and the fact that fetal waste products are largely metabolized in the placenta throughout gestation [8] lead us to think that the fetal kidney may be stimulated in development by maternal renal dysfunction.

We have previously showed several lines of evidence that maternal bilateral ureteral ligation stimulates the fetal development of the glomerulus [14] and proximal tubules [15], and suppresses the fetal growth [14]. The nature of such a compensatory stimulus, however, has not been clarified [1]. Goss [6] suggested that determining if fetal kidneys respond by compensatory growth to maternal uninephrectomy may provide information about the nature of such a compensatory stimulus. We have recently indicated that maternal uninephrectomy accelerates the fetal development of the glomerulus and proximal tubule by investigating the neonatal kidneys immediately after birth in the rat [16, 17].

Accordingly, the present study was designed to explore morphometrically whether fetal kidneys at various developmental stages could respond to a moderate and lasting stimulus induced by maternal uninephrectomy.

MATERIALS AND METHODS

Wistar rats were reared under ordinary conditions (24 ± 1°C, 14 hrs light) and given a commercial diet (NMF, Oriental Yeast Co., Tokyo, Japan) and water both *ad libitum*. The day following an overnight mating was counted as day 1 of gestation. The pregnant rats were divided into 2 groups: uninephrectomized and sham-operated. On day 5 of gestation, maternal uninephrectomy was performed by the method described in our previous report [16]. On days 18, 20, and 22 of gestation, fetuses were removed from the uterus under ether anesthesia and decapitated. Fetal left kidneys were removed, fixed in Bouin's solution, dehydrated

in a graded series of alcohol, and embedded in Tissue Prep (Fisher Scientific, New Jersey). Serial sections were prepared at 6 µm and stained with hematoxylin and eosin. The fetuses from uninephrectomized mothers were designated as E fetus and those from sham-operated ones as C fetus (Table 1).

Classification of glomerulus: Glomeruli in developing kidney were classified into 5 types by the modified criteria of our previous classification [14] as follows: Type I forms primitive renal vesicle (Fig. 1a); Type II, S-shaped corpuscle (Fig. 1b); Type III, a visceral layer of cuboidal epithelial cells and a parietal layer of Bowman's capsule (Fig. 1c); Type IV, more flattened visceral epithelial cells and more numerous capillary lumina than in Type III (Fig. 1d); and Type V, a structure similar to that of adult glomerulus (Fig. 1e).

Morphometry: Total glomerular volume of each type was determined by the following formula:

Total glomerular volume = glomerular volume × glomerular number per kidney

where glomerular volume was determined by the method of Eguchi and Morikawa [3] and glomerular number per kidney was determined by the method described in our previous paper [13].

Transsections of the proximal tubule were counted on every 30th section of the series of each kidney. Proximal tubular length per unit volume (1 mm³) of whole kidney (L_v) was calculated by the following formula [19]:

$$L_v = 2 \times Q_A$$

where Q_A is the ratio between the number of transections of the proximal tubule and the sectional area of the kidney used for counting. The proximal tubular length per kidney

Table 1. Age at surgery and autopsy in each uninephrectomized (E) and sham-operated (C) group

Group	No. of fetuses for morphometry	Day of surgery	Day of autopsy
E-18	8(3) ^{a)}	5	18
C-18	6(2)	5	18
E-20	8(3)	5	20
C-20	6(2)	5	20
E-22	5(3)	5	22
C-22	8(2)	5	22

a) No. of litters appear in parentheses.

(L_k) was calculated by multiplying the L_v by the renal volume estimated by the method used in estimating glomerular volume. The outer and inner diameters of the proximal tubule were determined with an ocular micrometer. The estimated proximal tubular cell height is a half of the distance between the two diameters. Both diameters were measured on 30 proximal tubular profiles per animal. Considering the proximal tubule as a cylinder [5], total proximal tubular volume was calculated by the following formula:

$$\text{Total proximal tubular volume} = \pi \times (\text{outer diameter}/2)^2 \times L_k$$

Statistical analysis of data was made with Student's *t* test. A *P* value of less than 0.05 was considered statistically significant.

RESULTS

Centrifugal growth pattern of the kidney was seen in fetuses: more mature glomeruli in deep cortex, less mature glomeruli in superficial cortex. In subcapsular area, there was a nephrogenic zone consisting of immature glomeruli and collecting tubules. On day 22 of gestation, the nephrogenic zone in E fetus was narrower than that in C fetus (Fig. 2a and b).

Morphometrical changes in proximal tubules are shown in Table 2. On day 18, no significant differences were observed in any kind of parameters used for the proximal tubule between E and C fetuses. On days 20 and 22, proximal tubular cell height was significantly lower in E fetus than in C fetus. Luminal diameter, length, and total volume of the proximal tubule were significantly larger in E fetus than in C fetus.

Changes in total glomerular volume of each type are shown in Table 3. On day 18, total glomerular volume of Type III in E fetus was significantly larger than that in C fetus. On day 20, total glomerular volume of Type V and the sum of volumes in E fetus were significantly larger than those in C fetus. On day 22, total glomerular volume of Type I in E fetus was significantly smaller than that in C fetus. Total glomerular volumes of Type II and V were

Fig. 1. a: Type I (renal vesicle). The vesicle is lined with columnar cells consisting of thick and thin walls. H.E. stain. $\times 450$. b: Type II (S-shaped corpuscle). The thick wall invaginates toward the thin wall, where capillary invasion occurs. H.E. stain. $\times 450$. c: Type III. Central part is covered by a single layer of cuboidal visceral epithelial cells with a large round nucleus. H.E. stain. $\times 450$. d: Type IV. Visceral epithelial cells are often squamous. Capillary lumina are seen more numerous than in Type III in Fig. 1c. H.E. stain. $\times 450$. e: Type V. Visceral epithelial cells are more squamous and capillary lumina are seen more numerous than in Type IV in Fig. 1d. H.E. stain. $\times 450$.

Fig. 2. a: Kidney of 22-day-old C fetus. Mature type of glomerulus is mostly seen in juxtamedullary cortical zone. Nephrogenic zone (N) consists of immature type of glomeruli and collecting tubules in subcapsular cortex. H.E. stain. $\times 110$. b: Kidney of 22-day-old E fetus. Nephrogenic zone (N) is narrower and profiles of the proximal tubule is more numerous than in the age-matched C fetus in Fig. 2a. H.E. stain. $\times 110$.

Table 2. Morphometrical changes in the proximal tubule in fetuses from uni-nephrectomized (E) and sham-operated (C) mothers (Mean $\pm$ SEM)

Group	Cell height (μm)	Luminal diameter (μm)	Length (mm)	Total volume (mm^3)
E-18	10.6 ± 0.1	6.9 ± 0.4	11.64 ± 1.09	0.72 ± 0.09
C-18	10.9 ± 0.2	7.1 ± 0.5	11.35 ± 1.93	0.76 ± 0.14
E-20	$9.3 \pm 0.1^*$	$15.0 \pm 0.4^*$	$182.84 \pm 12.91^*$	$16.16 \pm 1.25^*$
C-20	10.1 ± 0.1	13.1 ± 0.3	141.18 ± 9.91	12.22 ± 0.73
E-22	$9.1 \pm 0.2^*$	$17.5 \pm 1.0^*$	$1387.35 \pm 77.04^*$	$143.53 \pm 15.75^*$
C-22	10.0 ± 0.2	13.7 ± 0.5	1139.95 ± 58.65	101.24 ± 4.59

* Significantly different from C group ($p < 0.05$).

significantly larger than those in C fetus. No significant difference in G/P_v was observed between E and C fetuses at every stage examined.

DISCUSSION

Maturation of the kidney shows a centrifugal developmental pattern [7]; the nephrogenic zone appears in the subcapsular area, and the glomeruli in the superficial cortical zone are always smaller than those in the juxtamedullary zone [20]. The present result that the

nephrogenic zone became narrower in E fetus than in C fetus indicates that the development of fetal kidney is accelerated by maternal uninephrectomy.

In the proximal tubule, three distinct segments, S_1 , S_2 , and S_3 , are identified [4, 10]. S_1 comprises the beginning of the convoluted part, S_2 includes the end of the convoluted part and the beginning of the straight descending part, and S_3 is the remaining portion of the descending part [10]. During maturation, the epithelia of the proximal straight tubule and of thin descending limb of the loop of Henle differentiate from undifferentiated epithelium after the

Table 3. Total glomerular volume ($\times 10^{-2} \text{ mm}^3$) in each glomerular type and ratio of sum of volumes to total proximal tubular volume (G_v/P_v) (P_v shown in Table 2) in fetuses from uninephrectomized (E) and sham-operated (C) mothers (Mean $\pm$ SEM)

Group	Glomerular type					Sum	G_v/P_v
	I	II	III	IV	V		
E-18	0.72 $\pm$ 0.09	1.32 $\pm$ 0.10	0.48 $\pm$ 0.06*	0.12 $\pm$ 0.03	–	2.64 $\pm$ 0.24	3.850 $\pm$ 0.290
C-18	0.62 $\pm$ 0.13	1.50 $\pm$ 0.16	0.30 $\pm$ 0.05	0.13 $\pm$ 0.04	–	2.10 $\pm$ 0.31	3.360 $\pm$ 0.970
E-20	2.01 $\pm$ 0.23	2.59 $\pm$ 0.26	2.09 $\pm$ 0.15	2.46 $\pm$ 0.10	1.77 $\pm$ 0.21*	10.93 $\pm$ 0.49*	0.699 $\pm$ 0.052
C-20	1.72 $\pm$ 0.18	1.97 $\pm$ 0.23	1.87 $\pm$ 0.10	2.10 $\pm$ 0.27	1.05 $\pm$ 0.17	8.72 $\pm$ 0.81	0.707 $\pm$ 0.026
E-22	5.02 $\pm$ 0.81*	10.60 $\pm$ 0.83*	6.00 $\pm$ 0.73	8.48 $\pm$ 0.41	8.38 $\pm$ 0.54*	38.67 $\pm$ 2.61	0.286 $\pm$ 0.040
C-22	6.93 $\pm$ 0.34	6.01 $\pm$ 0.32	5.69 $\pm$ 0.35	9.40 $\pm$ 0.72	6.68 $\pm$ 0.48	34.70 $\pm$ 2.18	0.346 $\pm$ 0.019

* Significantly different from C group ($p < 0.05$).

formation of the proximal convoluted tubule [11]. Thus, the portions far from the glomerulus develop as the maturation of the kidney progresses. Further, cells in S_2 and S_3 are lower than those in S_1 [4, 10]. In the present study, by morphometrical analyses, the proximal tubular length was significantly longer in E fetus than in C fetus. Hence, the reduction in the proximal tubular cell height in E fetus on days 20 and 22 suggests that maternal uninephrectomy stimulates the formation of new segments (S_2 and S_3), rather than inducing changes in the cell height therein.

In developing kidney, Types IV and V of the glomerulus are functional in filtration, but Types I, II, and III are not [13]. In adult human, the variation of filtration surface area is essentially determined by that of total glomerular volume [12]. Therefore, the increases in the total glomerular volume of Type V on days 20 and 22 and in that of Type III on day 18 in E fetus suggest that maternal uninephrectomy induces a functional elevation in glomerular filtration on these days, but does not on day 18. Further, on fetal day 18, no significant differences were observed in any kind of parameters used for the proximal tubule between E and C fetuses. In contrast, on days 20 and 22, the luminal diameter, length and total volume of the proximal tubule in E fetus were significantly larger than those in C fetus. Thus, the stimulative effects of maternal uninephrectomy on morphological development of fetal proximal tubules are accompanied by changes in functional glomerulus. Since in perinatal rats, the onset of glomerular filtration induces the dilation of the tubular lumen in the loop of Henle [11], the elevated glomerular function during fetal period may stimulate the development of the proximal tubule.

As the number of Type II of the glomerulus in 1-day-old neonates born to uninephrectomized mothers is larger than that in neonates born to sham-operated mothers [16], the present finding that total glomerular volume of Type I was smaller and that of Type II was larger in E fetus than in C fetus on day 22 suggests that differentiation of the glomerulus from Type I to Type II is accelerated by maternal uninephrectomy.

The ratio of the sum of total glomerular volumes to the total proximal tubular volume (G_v/P_v) is one of the available

parameters in renal development, since glomerular maturation is accompanied by the appearance of defined renal tubules in developing kidney [18]. On the other hand, blood urea nitrogen (BUN) level in uninephrectomized non-pregnant rats is 1.5 times higher than that in control non-pregnant rats [16], while BUN level in bilateral ureter-ligated pregnant rats is 5 times higher than that in control pregnant rats [9]. BUN levels in fetal rats are similar to those in their mothers during pregnancy [9]. Maternal bilateral ureteral ligation decreases G_v/P_v in fetal kidney [15]. In the present study, however, no significant difference in G_v/P_v was observed between E and C fetuses at every stage examined. Based upon these findings, the present result of no difference in G_v/P_v may be explained by the fact that functional demand for the fetal kidney induced by maternal uninephrectomy is less than that induced by maternal bilateral ureteral ligation. Therefore, it is suggested that difference in degree of elevation of BUN level causes different stimulative effect on the fetal kidney. If maternal uninephrectomy causes no significant increase in BUN, the morphometrical changes in the fetal kidney could indicate that the stimulative effect of nephrectomy on the developing kidney is due to the reduction of maternal renal mass, but not due to the functional demand for fetal kidney. Uninephrectomy, however, causes significant increase in BUN level [16]. Therefore, whether elevated BUN level by nephrectomy stimulates the development of the fetal kidney directly or indirectly is remained unclear.

In summary, the present study clearly showed that maternal uninephrectomy stimulates the morphological development of the proximal tubule in fetuses in the rat.

ACKNOWLEDGMENT. This work was supported by Grant-in-Aid (No. 06760261) from the Ministry of Education, Science, Sports, and Culture of Japan.

REFERENCES

1. Austin, H. III, Goldin, H., and Preuss, H. G. 1986. Humoral regulation of renal growth: Evidence for and against the presence of a circulating renotropic factor. *Nephron* 27: 163–170.
2. Bakala, H., Geloso-Meyer, A., Cheignon, M., and

- Schaefferbeke, J. 1985. Differentiation of the glomerular filtration barrier in the rat fetus: possible role of collagen. *Connect. Tissue Res.* 13: 283–290.
3. Eguchi, Y. and Morikawa, Y. 1966. A study of the rat thyroid during perinatal days with observations of compensatory changes following unilateral thyroidectomy. *Anat. Rec.* 187: 147–152.
4. Fawcett, D. W. 1986. pp. 755–795. In: Bloom and Fawcett, A Textbook of Histology, 11th ed., W. B. Saunders, Philadelphia.
5. Fetterman, G. H., Shuplock, N. A., Philipp, F. J., and Gregg, H. S. 1965. The growth and maturation of human glomeruli and proximal convolutions from term to adulthood. Studies by microdissection. *Pediatrics* 35: 601–619.
6. Goss, R. J. 1963. Effects of maternal nephrectomy on foetal kidney. *Nature (Lond.)* 198: 1108–1109.
7. Kazimierzczak, J. 1971. Development of the renal corpuscle and the juxtaglomerular apparatus. A light and electron microscopic study. *Acta Pathol. Microbiol. Scand. Sect. A (Suppl. 218)*.
8. Liggins, G. C. 1972. The fetus and birth. pp. 77–109. In: Reproduction in Mammals, vol. 2, Embryonic and Fetal Development (Austin, C. R. and Short, R. V. eds.). Cambridge University Press, London.
9. Matsuo, M., Morikawa, Y., Hashimoto, Y., and Baratz, R. S. 1986. Changes in blood urea nitrogen (BUN) concentration during pregnancy in the rat with or without obstructive uremia. *Exp. Pathol.* 30: 203–208.
10. Maunsbach, A. B. 1966. Observation on the proximal tubule in the rat kidney. Comparison of results from phase contrast, fluorescence and electron microscopy. *J. Ultrastruct. Res.* 16: 239–258.
11. Neiss, W. F. 1982. Histogenesis of the loop of Henle in the rat kidney. *Anat. Embryol.* 164: 315–330.
12. Nyengaard, J. R. and Bendtsen, T. F. 1992. Glomerular number and size in relation to age, kidney weight, and body surface in normal man. *Anat. Rec.* 232: 194–201.
13. Okada, T. and Morikawa, Y. 1988. Histometry of the developing kidney in fetal rats. *Jpn. J. Vet. Sci.* 50: 269–272.
14. Okada, T. and Morikawa, Y. 1988. Effects of maternal bilateral ureteral ligation on the development of fetal kidney in rats: Histometrical study. *Jpn. J. Vet. Sci.* 50: 985–989.
15. Okada, T. and Morikawa, Y. 1990. Effects of maternal bilateral ureteral ligation on the development of the proximal tubule of the kidney in fetal rats: Morphometry and electron microscopic study. *Anat. Rec.* 228: 456–460.
16. Okada, T., Yamagishi, T., and Morikawa, Y. 1994. Morphometry of the kidney in rat pups from uninephrectomized mothers. *Anat. Rec.* 240: 120–124.
17. Okada, T., Yamagishi, T., Kiso, Y., Morikawa, Y., and Sasaki, F. 1995. Morphometry on the proximal tubule of the kidney in rat pups from uninephrectomized mothers. *J. Vet. Med. Sci.* 57: 415–417.
18. Potter, E. L. and Thierstein, S. T. 1943. Glomerular development in the kidney as an index of fetal maturity. *J. Pediatr.* 22: 695–706.
19. Seyer-Hansen, K., Gundersen, H. J. G., and Osterby, R. 1985. Stereology of the rat kidney during compensatory renal hypertrophy. *Acta Pathol. Microbiol. Immunol. Scand. Sect. A* 93: 9–12.
20. Zolnai, B. and Palkovits, M. 1965. Glomerulometrics II. New data concerning the growth of glomeruli in the albino rat. *Acta Biol. Hung.* 15: 393–408.