Perinatal ANG II programs adult blood pressure, glomerular number, and renal function in rats

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Woods, Lori L., and Ruth Rasch. Perinatal ANG II programs adult blood pressure, glomerular number, and renal function in rats. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R1593–R1599, 1998.—ANG II is known to be important in normal renal development, but the long-term consequences of a suppressed renin-angiotensin system (RAS) during the developmental period are not completely understood. This study tested the hypothesis that the RAS in the developing animal is important in long-term regulation of renal function and arterial pressure. Newborn Sprague-Dawley rat pups were given the ANG II AT1 receptor antagonist losartan (25 mg·kg·day−1·sc) for the first 12 days of postnatal life (Los). Body weights at weaning (22 days) were significantly reduced in Los (53.4 ± 3.2 vs. 64.5 ± 3.6 g in controls); however, at the time of study (~22 wk), body weights and the kidney-to-body weight ratios were not different. In chronically instrumented conscious animals, glomerular filtration rate and effective renal plasma flow were reduced by 27 and 20%, respectively, in Los; the filtration fraction was not different. Maximal urine concentrating ability was also reduced in Los (1,351 ± 6 vs. 2,393 ± 52 mosmol/kg in controls). Mean arterial pressure was significantly higher in Los (134 ± 3 vs. 120 ± 1 mmHg). The number of glomeruli per kidney was reduced by 42% in Los, but the total glomerular volume was unchanged. Thus perinatal blockade of ANG II AT1 receptors results in fewer but enlarged glomeruli, reduced renal function, and an increased arterial pressure in adulthood. These data indicate that perinatal ANG II, acting via AT1 receptors, plays an important role in renal development and long-term control of renal function and arterial pressure. Physiological conditions that cause suppression of the RAS in the developing animal may have long-term consequences for renal function and blood pressure.

glomerular filtration rate; renal plasma flow; losartan

THE RENIN-ANGIOTENSIN SYSTEM (RAS) has long been recognized to be an important regulator of arterial blood pressure in the adult mammal, including humans (11). ANG II participates in body fluid volume and blood pressure regulation by causing generalized vasoconstriction, stimulating renal retention of salt and water, and increasing thirst. Although probably the best-known stimulus for the RAS in the adult is salt (Na+) restriction, dietary protein intake is also known to alter this system. Protein loading activates the RAS, whereas protein restriction suppresses it (30). Recent evidence also supports a role for ANG II as a growth factor in at least some adult tissues (22, 25).

Numerous studies suggest that the RAS is also important in the mammalian fetus and that the major mechanisms known to stimulate renin release in the adult are functional before birth (7, 28, 31, 32). Indeed, the RAS appears to be particularly active during the perinatal period. Given the known role of ANG II as a growth factor in the adult, it is not surprising that emerging evidence suggests an important role for the RAS in the developing animal. In particular, several studies have shown that ANG II plays a vital role in the structural development of the kidney (6, 37). Despite these findings, however, the role of the perinatal RAS in long-term control of renal function is not well understood. Moreover, although the adult kidney is known to be the major long-term controller of arterial blood pressure, the implications of the role of the perinatal RAS in renal development for long-term control of arterial blood pressure are largely unknown. The purpose of these studies was to test the hypothesis that the RAS in the developing animal plays an important role in long-term regulation of renal function and arterial pressure.

METHODS

Female Sprague-Dawley rats (Simonsen) weighing ~250–300 g were bred at Oregon Health Sciences University and maintained on a normal-protein (19%), normal-sodium (0.20%) diet (Purina basal diet 5755) ad libitum throughout pregnancy and lactation. Newborn pups (6 females and 3 males) were treated with the ANG II AT1 receptor antagonist losartan (25 mg·kg·day−1·sc) for the first 12 days of postnatal life. Nine gender-matched littermate controls were given saline vehicle. Pups were weaned to the above diet at 22 days of age and maintained on that diet until instrumentation. The animals were housed in a room with a controlled temperature and a 12:12-h light-dark cycle.

Surgical preparation of adult animals. At ~21 wk of age, the adult animals were anesthetized with a mixture of 55% ketamine (100 mg/ml), 28% xylazine (20 mg/ml), 11% acepromazine (10 mg/ml), and 6% sterile water, administered at 1.0 ml/kg ip. The sites of the incisions were shaved and swabbed with betadine, and all catheters, suture, instruments, and gloves used were sterile. Through a midline abdominal incision, a stainless steel Silastic-covered catheter was inserted through a puncture hole at the apex of the bladder and secured by a purse string suture. The catheter exited on the ventral surface of the abdomen, and the muscle and skin were sutured closed around it. The bladder was flushed with chloramphenicol sodium succinate (30 mg/ml), and the catheter was plugged with a stainless steel pin covered with Silastic tubing. Sterile catheters made of Tygon microbore tubing were implanted into the left femoral artery...
Inulin (Sigma, St. Louis, MO) and p-aminohippurate (PAH) (Sigma) in 5% dextrose were given intravenously as a bolus (0.45 ml containing 56 mg inulin and 5.6 mg PAH) followed by a continuous infusion (0.024 ml/min of 74 mg/ml inulin and 7.4 mg/ml PAH) throughout the rest of the experiment. At least 60 min after the beginning of the inulin-PAH infusion, three or four successive 20-min urine collections (clearance periods) were done, with a blood sample obtained after each. Urine volume was determined gravimetrically.

Experimental protocol. The animals were 22 ± 1 wk of age at the time of study. On the days on which physiological measurements were made, the rat was placed in a wire restrainer in the study room. The plug of the bladder catheter was removed, and urine was allowed to drain continuously into a tube throughout the experiment. Mean arterial pressure was measured through the arterial catheter using a pressure transducer (Statham, Oxnard, CA) connected to a polygraph (Grass Instruments, Quincy, MA), and a reading was taken after at least 30 min, once the pressure had stabilized. Arterial pressures were always measured between 6:00 and 9:00 AM. A small blood sample was taken from the arterial catheter for measurement of microhematocrit and plasma protein. Inulin (Sigma, St. Louis, MO) and p-aminohippurate (PAH) (Sigma) in 5% dextrose were given intravenously as a bolus (0.45 ml containing 56 mg inulin and 5.6 mg PAH) followed by a continuous infusion (0.024 ml/min of 74 mg/ml inulin and 7.4 mg/ml PAH) throughout the rest of the experiment. At least 60 min after the beginning of the inulin-PAH infusion, three or four successive 20-min urine collections (clearance periods) were done, with a blood sample taken at the midpoint of each. Blood was collected in sterile heparinized syringes. Urine volume was determined gravimetrically. After centrifuging the blood and removing the plasma, we resuspended the red blood cells in an equivalent volume of saline and returned them to the animal. The plug of the bladder catheter was removed, and the bladder was allowed to empty. Two successive samples of newly formed urine were collected by allowing urine to drain into tubes through the bladder catheter. The animals were then returned to their cages and allowed access to water for 1–2 days before they were euthanized.

When all experiments were completed or when the instrumentation was no longer functional, the rats were killed with a commercial euthanasia solution. The left kidney was fixed in 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate, cut in 2-mm-thick horizontal slices, and embedded in Technovit. From each slice, five serial sections (15 µm) were cut and stained with periodic acid Schiff and hematoxylin. Two sections 15 µm apart were used for the disector measurements, making the thickness for the disector calculations 30 µm. The total glomerular volume per kidney was measured by point counting on the kidney sections: $V_{glomer} = V_{glomer} \times V_{kid}$, where $V_{glomer}$ is the glomerular fraction per kidney and $V_{kid}$ is the kidney volume. Glomerular number was determined on complete sections by applying the disector method (23). This method counts particles by means of randomly selected serial section pairs of known thickness. The glomerular profiles are sampled by means of the unbiased two-dimensional counting rule (34). Glomerular profiles are counted if they are present in one of the serial sections and not in the other. The number of glomeruli is calculated as $N_{glomer} = Q / (B \times a_{cyl} \times V_{kid})$, where $Q$ is the number of glomerular profiles counted, $B$ is the section thickness, $a_{cyl}$ is the area counted, and $V_{kid}$ is the kidney volume. The average volume of one glomerulus, $V_{glomer}$, was estimated from the measurements of the total glomerular volume per kidney and the glomerular number: $V_{glomer} = V_{glomer}/N_{glomer}$.

The right kidney was fixed in 10% phosphate-buffered Formalin and embedded in paraffin. Sections were stained with hematoxylin and eosin and used for evaluation of renal pathology.

Analytic measurements. Inulin in plasma and urine was assayed by a modification of the method of Waugh (38) after deproteinization with zinc sulfate. PAH was assayed on the same samples using the method of Brun (3). Glomerular filtration rate (GFR) was calculated as the renal clearance of inulin [GFR = $\left(U_{in} \times P_{in}\right) / V$], where $U_{in}$ and $P_{in}$ are the urine and arterial plasma inulin concentrations, respectively, and $V$ is the urine flow rate. Effective renal plasma flow (ERPF) was calculated as the renal clearance of PAH. The values obtained for the three or four clearance periods were averaged to give a single value for each animal. Urine osmolality was measured by freezing point depression (Advanced Instruments, Needham Heights, MA). Plasma protein was measured by refractometry (National Instrument, Baltimore, MD).

Statistical analysis. The data are expressed as means ± SE. Data for the two groups were compared using a paired t-test. Statistical significance was assumed with a value of $P < 0.05$ or better.

RESULTS

Effects of perinatal AT1 receptor blockade on growth. Body weights at weaning were significantly reduced in losartan-treated rats compared with controls (64.5 ± 3.6 g in control vs. 53.4 ± 3.2 g in losartan); however, body weights at the time of study were not different (334 ± 35 g in control vs. 337 ± 31 g in losartan). Mean arterial pressure was significantly increased by 10.2 ± 0.3 mmHg in control vs. 20.0 ± 0.17 mmHg in losartan. Thus perinatal blockade of AT1 receptors appears to impair postnatal growth but does not have a lifelong effect on body or kidney weight.

Effects of perinatal AT1 receptor blockade on physiological variables. Hematocrits (38 ± 1%) and plasma protein levels (6.6 ± 0.1 g/dl) were not different between the two groups. Arterial pressures and renal hemodynamics in control adult rats and adult rats treated perinatally with losartan are shown in Fig. 1. Arterial pressure was significantly increased by 13 ± 2 mmHg in losartan-treated rats compared with controls. GFR was significantly reduced by an average of 27%, and ERPF, by 20%. However, the mean filtration fraction was not significantly different between the two groups (34 ± 2% in control vs. 31 ± 1% in losartan). The GFR and ERPF normalized to either kidney or body weight (not shown) were also significantly reduced in losartan-treated animals. Maximal urine concentrating ability was also significantly reduced in losartan-
Effects of perinatal AT$_1$ receptor blockade on renal structure. Grossly, kidneys of rats treated perinatally with losartan had a definable granularity on the surface, compared with a smooth surface in kidneys of control animals. On gross inspection, losartan-treated animals appeared to have smaller renal papillae. There was no evidence of obstruction or other abnormalities of the lower urinary tract. Representative photomicrographs of right kidneys of adult control animals and adult animals treated perinatally with losartan are shown in Fig. 2. The kidneys of losartan-treated rats showed focal tubular collapse and atrophy, mainly involving segments of the proximal tubule, and associated local fibrosis and infiltrates of lymphocytes. There were also scattered clusters of dilated tubule segments at all levels, including the outer medulla, that rarely contained neutrophils. Glomerular collapse and segmental sclerosis were seen in rare instances. Arterial medial thickening, most evident in the cortical radial and arcuate arteries, was also present.

The total number of glomeruli and glomerular volume are shown in Fig. 3. Total glomerular number was significantly reduced by 42% in losartan-treated animals, and the average volume of an individual glomerulus was increased by 53%. Thus the total volume of all glomeruli was not different from that in the control animals.

**DISCUSSION**

The most important findings of the present study are that blockade of the angiotensin AT$_1$ receptor during the immediate postnatal period (latter portion of the period of nephrogenesis) in the rat resulted in an increased arterial pressure, a decreased number of glomeruli, and decreased renal function in adulthood. Thus this study demonstrates for the first time that the RAS in the immediate perinatal period plays an important role in long-term regulation of arterial pressure as well as renal structure and function.

Importance of RAS in renal development. A number of studies using antagonists of the RAS have indicated that ANG II plays an important role in nephron development (5, 6, 37). Notably, angiotensin-converting enzyme (ACE) inhibitors or specific AT$_1$ but not AT$_2$ blockade in neonatal rats of several strains produces renal vascular and tubular histological abnormalities (6, 37). ACE inhibition also resulted in fewer and smaller glomeruli in the mesonephric kidney of the frog, a model of the embryonic kidney (37). Results from transgenic animals deficient for genes of the RAS, including angiotensinogen (21), ACE (12), and AT$_1A$ (26), have also generally pointed to an important role for the RAS in renal development. As progress in this field continues, it seems likely that studies using gene-targeting approaches will also continue to support this important function of the RAS.

Perinatal RAS and long-term physiological regulation. Although numerous studies using both pharmacological and gene-targeting approaches indicate that the actions of ANG II in the perinatal period are vital for normal renal histological development, the importance of the perinatal RAS in long-term control of renal function and arterial pressure is largely unknown. To our knowledge, only one group has addressed this question (6, 9). They found that rats treated neonatally (from 3–24 days of age) with the converting enzyme inhibitor enalapril had reduced urine concentrating ability, GFR, and ERPF and histological abnormalities including papillary atrophy, interstitial fibrosis and inflammation, and tubular atrophy and dilatation (9). Rats treated with losartan over a similar time period showed papillary atrophy and an increased free-living urine volume with reduced urine osmolality, whereas rats treated with an AT$_2$ receptor antagonist did not (6). These previous findings are consistent with our present results.

The major functional difference between our results and those in the previous study is that arterial pressure was strikingly and significantly elevated in conscious rats treated perinatally with losartan, whereas there was no difference in arterial pressure between anesthetized enalapril- and vehicle-treated rats (9). Additionally, we also found that structurally, the number of glomeruli in kidneys of losartan-treated rats was significantly reduced, which apparently was not noted after neonatal treatment with enalapril. The reason for these discrepancies is not clear, but they may be due at least in part to two important differences in experimen-
tal design: the specific actions of the blocker used and the time period over which the animals were treated. In particular, the effects of ANG II acting via AT1 and AT2 receptors may be reciprocal in nature, so that simultaneous blockade of both pathways with a converting enzyme inhibitor may obscure separate functions of the two receptors, whereas specific blockade of one receptor subtype may unmask a role of the other. Thus ANG II

Fig. 2. A: renal cortex of a control animal at low magnification. Architecture is entirely preserved (×64). B: renal cortex of a losartan-treated (Los) animal at low magnification. Focal tubular collapse and segmental dilatation are evident, resulting in indentation of overlying surface contour (×64). C: renal cortex of a control animal at medium magnification showing no evidence of tubulointerstitial or glomerular abnormalities (×200). D: renal cortex of a Los animal at medium magnification. Focal tubular collapse is accompanied by mononuclear inflammatory infiltrates. A few tubule segments are dilated (×200). All sections stained with hematoxylin and eosin.
may normally promote renal growth and differentiation during development through its actions at the AT1 receptor, whereas its actions at the AT2 receptor may normally retard or inhibit this effect. Therefore, one long-term role of the perinatal AT1 receptor may be to promote formation of glomeruli and to "program" a normal blood pressure set point; the AT2 receptor may serve to limit these effects. If this is the case, blockade of the AT1 receptor alone would be expected to result in a reduced number of glomeruli and an increased blood pressure, whereas blockade of ANG II production, affecting both AT1 and AT2 pathways, might have little effect on either variable. This explanation would be consistent with both our findings and those of previous studies. It is also possible that blockade of the AT1 receptor may have resulted in higher-than-normal levels of ANG II and thus increased stimulation of the possibly inhibitory AT2 receptor. Although final resolution of this issue will await additional studies in which the AT2 receptor is blocked, alone as well as in concert with blockade of the AT1 receptor, it is clear from the present work that the perinatal RAS plays an important role in long-term regulation of arterial pressure.

Another possible explanation for the increased arterial pressure in our losartan-treated animals and the absence of hypertension in enalapril-treated animals involves the timing of treatment. It was our goal in the present study to specifically target the period of nephrogenesis, which normally is completed by ~10 days of postnatal age in the rat (17) (in contrast, the human fetus normally has its full complement of nephrons by 36 wk of gestation, i.e., before birth; see Ref. 4). Thus we administered the blocker from the day of birth until the pups were 11 days old. In contrast, in the previous studies, enalapril was given from 3 to 24 days of age (until approximately the age of weaning). Our animals were therefore treated only during the period of nephrogenesis, whereas those in the earlier studies were treated both during and after the nephrogenic period. Administration of losartan to spontaneously hypertensive rat pups between postnatal days 10 and 20 has been reported to reduce their adult blood pressures (15). It is possible that RAS blockade during nephrogenesis has an effect to increase the future blood pressure set point, whereas blockade after nephrogenesis has effects to decrease that set point. If so, the combined effects of treatment both during and after nephrogenesis might be expected to cancel each other out, resulting in no difference in arterial pressure in adulthood, as reported by Guron et al. (9).

Renal structural effects of perinatal AT1 receptor blockade. We found that the number of glomeruli was reduced in losartan-treated animals, suggesting that AT1 receptor blockade may have halted nephrogenesis, which is only partially complete at birth in the rat. Thus the glomeruli that are present in the adult losartan-treated animals may be those in which development was completed before losartan treatment was begun. The average individual glomerular volume increased, compensating for the loss in number, such that the total glomerular volume was not different in the two groups. This finding is similar to that in other situations in which the total number of glomeruli is reduced. In animals uninephrectomized immediately after birth and studied in parallel with those in the present study, we have found that perinatal surgical reduction of glomerular number causes an approximate doubling of the average glomerular volume (Woods and Rasch, unpublished results). Glomeruli of animals uninephrectomized in adulthood are also known to increase their size in response to the surgical reduction in number (20).

In contrast to our findings, another study has reported that histopathological renal lesions were associated only with prenatal, and not postnatal, treatment of rat pups with losartan (33). However, that study is not directly comparable to the present one because losartan was administered to the pregnant or lactating dams rather than to the pups themselves.

Relationship between reduced number of glomeruli and hypertension. We believe that our two important new findings of hypertension and reduced number of nephrons (glomeruli) in rats treated perinatally with losartan are causally related. The kidney has long been known to play a key role in long-term regulation of arterial pressure (10). Over the past several years, Brenner and colleagues (2) have postulated that the risk of developing essential hypertension in adulthood is inversely related to the nephron endowment at birth. Evidence in support of this hypothesis is that hypertension is more prevalent in human populations that have
smaller kidneys (19, 35, 36) and that inbred rat models of hypertension have fewer nephrons than their respective controls (2). A recent preliminary report also suggests that the number of nephrons in spontaneously hypertensive rats by transplanting a third kidney into them reduces their blood pressure compared with sham-operated animals (27). We have recently shown that surgical reduction in the number of nephrons (uninephrectomy), when done during development, results in hypertension in adulthood (39). Indeed, the magnitude of this increase in blood pressure was similar to that seen in the present study in losartan-treated animals, and the magnitude of the decrease in the number of nephrons was also similar. Thus it seems likely that the reduced number of glomeruli in rats treated perinatally with losartan may contribute to their increased adult blood pressure.

Perspectives

The results of the present studies may have profound implications for human health and disease. Epidemiologic evidence indicates that babies that are born smaller or who grow more slowly during the first year of life have an increased incidence of hypertension and death from cardiovascular disease when they reach adulthood (1, 18). This suggests that some factor(s) in the perinatal environment, probably related to maternal nutrition, can program the individual for increased cardiovascular risk later in life. The present studies suggest a possible physiological mechanism that could contribute to this perinatal programming, namely suppression of the fetal/neonatal RAS by maternal dietary factors. Indeed, in the rat, maternal dietary protein restriction during pregnancy leads to a suppressed RAS in newborn offspring (14) and a reduced number of nephrons (40) and hypertension in adult offspring (16). The results of the present studies suggest that these independent findings may be linked in a cause-and-effect manner. In other words, perinatal suppression of the RAS in offspring of protein-restricted mothers may lead to a reduced number of nephrons and consequent hypertension in adulthood. Our data also suggest that other physiological conditions that alter the RAS in the developing baby could have important long-term consequences for renal function and blood pressure.

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