Single Nephron Hemodynamics in Spontaneously Hypertensive Rats (43894)

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Abstract. **This study was designed to determine whether glomerular hypertension develops as a function of age in the spontaneously hypertensive rat (SHR). Male SHR and age-matched Wistar-Kyoto (WKY) normotensive controls were divided into three groups for measurements of whole kidney and single nephron hemodynamics at 5, 10, and 15 months of age. As reported previously, SHR developed significant proteinuria which was predominantly an albuminuria, after 5 months of age. There were no differences in whole kidney or single nephron glomerular filtration rates between SHR and WKY. Afferent glomerular capillary hydraulic pressure (P_{GC}) was slightly increased in SHR compared with WKY at 10 months of age. At 15 months of age, P_{GC} in SHR was significantly lower than WKY. Our studies indicate that increased capillary pressure is not a major factor in the development and progression of renal injury in the spontaneously hypertensive rat.** [P.S.E.B.M. 1995, Vol 209]

The spontaneously hypertensive rat (SHR) develops progressive functional and histopathological changes in the kidney which are unrelated to systemic blood pressure (1–3). These changes begin with a minimal increase in urinary albumin excretion at about 5 months of age and rapidly accelerate to moderate proteinuria and glomerular sclerosis after 10 months of age. With the use of antihypertensive drug therapy, the life span of the SHR is markedly prolonged from 15 to 24 months of age (2, 3). Despite the improved longevity provided by normalized systemic blood pressure, the onset and magnitude of proteinuria and glomerular sclerosis is only delayed, not prevented.

Studies in other models of experimental renal disease have implicated increased glomerular capillary pressure (glomerular hypertension) as a pathogenetic factor in progressive glomerular injury (4–7). In the SHR, measurements of glomerular capillary pressure are comparable to normotensive, age-matched controls (8–11). However, these studies of glomerular hemodynamics in the SHR were only performed up to 6–8 months of age. The present study was undertaken to determine whether glomerular hypertension occurs in the aging SHR at 10 and 15 months of age.

Materials and Methods

Experimental Protocol. Male SHR (n = 25) and Wistar-Kyoto (WKY) normotensive controls (n = 27) were obtained from Harlan Sprague Dawley (Indianapolis, IN) at 3 months of age. They were maintained on standard laboratory pellet chow (24% protein, Teklad, Madison, WI). SHR and WKY rats were divided into three groups for measurements of whole kidney and single nephron hemodynamics at 5, 10, and 15 months of age.

Rats were housed in metabolism cages with free access to water. Overnight (18 hr) urine collections were performed 3–5 days prior to micropuncture studies. Systolic blood pressure was determined by the tail-cuff method (Narco Biosystems, Houston, TX).

Measurement of whole kidney and single nephron hemodynamics have been previously described (12). Rats were anesthetized with Inactin (Promonta; 80–100 mg/kg body wt, intraperitoneally) and placed on a heated table to maintain rectal temperature at 37°–

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38°C. After the animals were anesthetized, a midline neck incision was made, and the trachea was isolated and cannulated. Catheters were inserted into the left jugular vein for infusion during the experiments, the right internal carotid artery for blood pressure monitoring and blood sampling, and the bladder for urine collections. A left subcostal abdominal incision was made to expose the kidney, which was placed in a lucite cup, immobilized with agar, and bathed with warm isotonic saline. An intravenous infusion of isotonic saline solution with synthetic inulin (polyfructosan, 4 g/dl), 1 mCi/ml (specific activity 42 mCi/g) of [methoxy-^{3}H] inulin final concentration of isotope 0.06 mCi/ml; New England Nuclear, Boston, MA) and para-aminohippuric acid (PAH, 0.1 g/dl) was given at a rate of 0.06 ml/min. After 30–45 min of equilibrium, two clearance periods of approximately 60 min were carried out in each experiment. Blood pressure was monitored continuously from the left internal carotid artery by using a Gould P32ID transducer (Gould Inc., Oxnard, CA) and monitor (model SP 1405).

An average of five samples of tubule fluid were collected quantitatively from the late proximal convoluted tubule sites for determination of single nephron glomerular filtration rate. These sites in proximal tubules were identified when the oil droplet reappeared once or not at all. Collections were taken over a period of 3–4 min after an oil block was established with a 10–12 μm tip pipette filled with Sudan black stained castor oil. The sample volume (20–40 nl) was determined from its measured length in a constant bore capillary tube. After early segments of proximal tubules were identified by injection of small amounts of isotonic saline stained with FD & C green, free flow pressure and stop flow pressure in the same tubule were measured with a 3–7 μm micropipette connected to servonull micropressure system (World Precision Instruments, Sarasota, FL). An average of four pressure estimates were made. Stop flow pressures were determined by blocking the proximal tubule with Sudan black stained castor oil contained in a 12–14 μm tip micropipette. Values for each rat were reduced to a mean value for statistical analysis. In a limited number of experiments (n = 17) at 10 and 15 months, a renal vein sample was taken with a 30-gauge needle at the end of the second clearance period to determine the extraction ratio for PAH.

Analytical Methods. A microcontinuous gradient gel electrophoresis procedure was used for the separation and quantitation of proteins, including albumin, in plasma (diluted 1/51) and urine (1). Tritiated samples of plasma, urine, and tubule fluid were placed in 10 ml of Ecolume scintillation fluor (ICN Biomedical Inc., Irvine, CA) and counted in a Packard Tri-Carb scintillation counter. PAH concentrations in urine and plasma were analyzed according to the method of Smith *et al.* (13). The mean plasma concentration was 1.0 mg/dl and was therefore well below the concentration at which a secretory maximum is reached (Tm_{PAH}) (14).

Calculations. Afferent glomerular capillary hydraulic pressure (P_{GC}) was determined indirectly from the sum of stop flow pressure (SFP) and plasma colloid osmotic pressure. The colloid osmotic pressure of the arterial plasma (πA) was determined by the following equation (15):

$$\pi = 1.886C + 0.206C^2 + 0.005C^3$$

in which C is the plasma protein concentration as determined by electrophoresis (1). The glomerular transcapillary hydraulic pressure difference (ΔP) was calculated as the difference between P_{GC} and free flow pressure (FFP). In selected experiments renal plasma flow rate (RPF) was estimated from the clearance and extraction ratio of PAH. Whole kidney filtration fraction was estimated from GFR/RPF.

Statistics. Results are expressed as mean ± SEM. SHR and WKY rats were compared using a Student's t test for differences between WKY and SHR. Changes in renal function with age in both groups were evaluated using a one-way analysis of variance followed by a Tukey B test. A P value of less than 0.05 was considered significant. Statistical analysis was performed using Statistical Program for Social Science (SPSS, Chicago, IL).

Results

Urinary protein and albumin excretion were significantly different in the two groups at all ages (Fig. 1). Based on electrophoretic patterns of urinary proteins, the increasing importance of the albumin fraction is evident after 5 months of age. At 15 months of age, the albumin percentage was 45 ± 2% in the SHR compared with 17 ± 2% in the WKY controls.

Mean arterial blood pressure was significantly greater in SHR as compared with normotensive controls (Table I). Whole kidney glomerular filtration rate was similar at all ages between SHR and WKY groups. Effective renal plasma flow rate estimated by the clearance of PAH was significantly lower in the SHR compared to WKY at 5 months and 15 months of age. At 15 months of age renal plasma flow was also lower in SHR and resulted in a filtration fraction of 0.36.

Single nephron glomerular filtration rate was similar in the two groups (Table II) expressed as nl/min or nl/min/g kidney weight. Both free flow and stop flow pressures were nearly identical in both groups at each study period. There was a modest elevation in glomerular capillary pressure at 10 months and a fall at 15 months in the SHR group which represents a small change in colloid osmotic pressure. Glomerular trans-

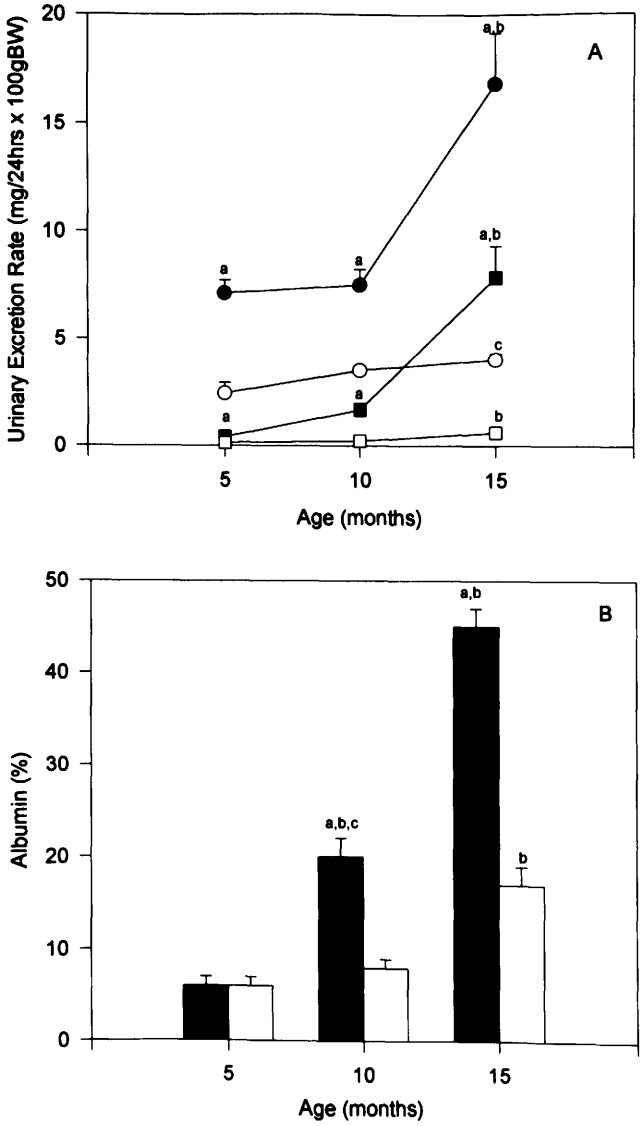

Figure 1. (A) Urinary protein and albumin excretion versus age. Circles represent protein, and squares represent albumin excretion. Closed (●■) symbols are SHR and open (○□) symbols are WKY. (B) The albumin contribution as a percent of total protein. Closed bars are SHR, open bars are WKY. [a]Significantly different from age-matched WKY at $P < 0.05$; [b] significantly different from values at 5 and 10 months at $P < 0.05$; [c] significantly different from values at 5 months at $P < 0.05$. The number of animals for each age were: WKY: 5 months, $n = 6$; 10 months, $n = 11$; 15 months, $n = 10$. SHR: 5 months, $n = 5$; 10 months, $n = 10$; 15 months, $n = 10$.

capillary hydraulic pressure was not different between SHR and WKY at 10 or 15 months.

Discussion

We previously defined the pattern of natural history of renal damage in untreated SHR (1). At about 5–6 months of age, there is a significant, although small, increase in urinary albumin excretion. After 8 months of age, proteinuria rapidly increases and is associated with significant glomerular, vascular, and interstitial damage. It is apparent that increased glomerular capillary pressure in cortical nephrons is not implicated in the initiation or progression of renal injury in the spontaneously hypertensive rat.

At 15 months of age, the filtration fraction in the SHR increased due to a significant decrease in renal plasma flow. The reason for the high filtration fraction is unclear and requires additional investigation.

Previous glomerular hemodynamic studies in the spontaneously hypertensive rat have revealed no elevation in glomerular capillary pressure in cortical nephrons. Azar *et al.* (9) and Arendshorst and Beierwaltes (8) found no difference in glomerular capillary pressure, stop flow pressure, single nephron glomerular filtration rate, or single nephron blood flow between SHR and WKY controls at 3–4 months of age. Glomerular hemodynamics performed at 10 months of age have reported a small increase of glomerular capillary pressure in superficial cortical glomeruli in SHR (11), although these elevated pressures are comparable to the WKY values in our study. In studies using the uninephrectomized SHR and WKY, Bank *et al.* (10) observed no increase in glomerular capillary pressure in superficial cortical glomeruli. However, there was a disproportionate increase in single nephron glomerular filtration rate in juxtamedullary nephrons. Despite the larger filtration rates in juxtamedullary compared with superficial nephrons, there is no evidence that juxtamedullary nephrons are exposed to higher systemic pressures leading to selective glomerular damage observed in our studies (1, 2). In fact, Jamison *et al.* have calculated that even an excess of 30 mm Hg of juxtamedullary over superficial net filtration pressure is insufficient to account for the larger filtration rate in juxtamedullary glomeruli (16).

We previously reported that juxtamedullary glomeruli are the first population of nephrons to demonstrate histopathological damage. This population is structurally and functionally different from superficial nephrons in regard to size (16, 17), diameter of efferent arterioles (18, 19), and origin of the afferent arteriole from the interlobular arteries. Recent studies have shown that the balance between afferent and efferent resistances in the SHR favors preglomerular vasoconstriction and the maintenance of normal superficial cortical intraglomerular capillary pressure. Kimura and collaborators using the vascular cast method found the ratio of diameters of afferent to efferent arteriole was about 25% lower in SHR than those in WKY at 5 months of age (20, 21). The lower arteriolar diameter ratio was due to afferent constriction and efferent dilatation. This ratio was similar in representative samplings from superficial and deeper cortical areas. Further support for afferent arteriolar vasoconstriction is based on vascular reactivity studies. Ito *et al.* employed an *in vitro* system to microperfuse afferent arterioles in the SHR in order to assess pressure-

Table I. Whole Kidney Function

Group	Age	Body wt (g)	Kidney wt (g)	GFR (ml/min × g KW)	C_{PAH} (ml/min × g KW)	MAP (mm Hg)
WKY	5 months (n = 6)	314 ± 5	2.45 ± 0.18	0.56 ± 0.05	2.81 ± 0.23	118 ± 4
	10 months (n = 11)	363 ± 7[a]	2.40 ± 0.25	0.96 ± 0.09	2.77 ± 0.36	120 ± 5
	15 months (n = 10)	390 ± 8[b]	2.66 ± 0.09	0.76 ± 0.09	2.72 ± 0.18	114 ± 3
SHR	5 months (n = 5)	327 ± 6	2.08 ± 0.08	0.74 ± 0.08	2.24 ± 0.12[c]	172 ± 8[c]
	10 months (n = 10)	388 ± 8[a,c]	2.49 ± 0.06[a]	0.85 ± 0.08	3.23 ± 0.36	172 ± 3[c]
	15 months (n = 10)	423 ± 7[b,c]	3.02 ± 0.08[b,c]	0.67 ± 0.15	1.51 ± 0.31[c,d]	153 ± 6[b,c]

Note. GFR = glomerular filtration rate; C_{PAH} = clearance of PAH; MAP = mean arterial pressure.
[a] Significantly different from values at 5 months at $P < 0.05$; [b]significantly different from values at 5 and 10 months at $P < 0.05$; [c]significantly different from age-matched WKY at $P < 0.05$.

Table II. Single Nephron Function

Group	Age	SNGFR (nl/min × g KW)	FFP (mm Hg)	SFP (mm Hg)	P_{GC} (mm Hg)	ΔP (mm Hg)	CA (g/dl)	πA (mm Hg)
WKY	5 months (n = 6)	27.5 ± 2.3	13 ± 1	30 ± 1	45 ± 1	33 ± 1	5.0 ± 0.2	15 ± 1
	10 months (n = 11)	31.5 ± 2.2	13 ± 1	28 ± 1	43 ± 1	30 ± 1	5.0 ± 0.2	15 ± 1
	15 months (n = 10)	32.9 ± 2.4	14 ± 1	30 ± 1	45 ± 1	30 ± 1	5.1 ± 0.3	15 ± 1
SHR	5 months (n = 5)	28.5 ± 3.6	13 ± 1	30 ± 1	46 ± 1	33 ± 1	5.3 ± 0.1	16 ± 1
	10 months (n = 10)	33.5 ± 2.6	14 ± 1[a]	31 ± 1[a]	47 ± 1[a]	34 ± 1	5.3 ± 0.1	17 ± 1
	15 months (n = 10)	30.9 ± 4.0	14 ± 1	30 ± 1	42 ± 1[a,b]	29 ± 1	4.3 ± 0.1[b]	12 ± 1[b]

Note. SNGFR = single nephron glomerular filtration rate; FFP = free flow pressure; SFP = stop flow pressure, P_{GC} = afferent glomerular capillary hydraulic pressure; ΔP = glomerular transcapillary hydraulic pressure difference; CA = afferent protein concentration; πA = colloid osmotic pressure.
[a] Significantly different from age-matched WKY at $P < 0.05$; [b]significantly different from values at 5 and 10 months at $P < 0.05$.

induced arteriolar luminal diameter (22). When perfusion pressure increased to greater than 100 mm Hg, the diameter of afferent vessels in SHR decreased significantly compared to WKY. These studies have shown that the diameters of the preglomerular vasculature are reduced secondary to elevated vascular tone (22, 23). Based on these *in vitro* studies, vasoconstriction of preglomerular arterioles of superficial and juxtamedullary cortical glomeruli should protect the glomeruli from increased systemic blood pressure. The end result would be normal glomerular capillary pressure and limited pathological damage. Our studies (1, 2) are in agreement with this explanation for superficial glomeruli. However, the afferent arteriolar vasoconstriction of juxtamedullary glomeruli is inconsistent with the findings of severe juxtamedullary glomerular and interstitial injury (1, 2, 24). Since direct measurements of juxtamedullary glomerular capillary pressures are not possible in the intact kidney, it is still very plausible that increased pressures and/or flow are transmitted to this population of nephrons compared with the superficial population (1, 2, 24).

In conclusion, our studies suggest that increased superficial cortical glomerular capillary pressure is not a major factor in the development and progression of renal injury in the spontaneously hypertensive rat. Additional investigations are necessary to understand the predilection for the localized, early juxtamedullary damage in the SHR kidney.

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