Regulation of glomerular capillary pressure and filtration rate in young Kyoto hypertensive rats

S. AZAR, M. A. JOHNSON, J. SCHEINMAN, L. BRUNO AND L. TOBIAN
Departments of Internal Medicine and Pediatrics, University of Minnesota Hospital and School of Medicine, Minneapolis, Minnesota, U.S.A.

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Summary
1. Single nephron pressures, flows and resistances were studied in 17–18 week old genetically hypertensive and normotensive Kyoto rats resembling one another in renal weight, total filtration rate, morphology and number of glomeruli in the kidney.

2. Glomerular capillary pressures were similar in the two groups; however, the hypertensive rats had lower pressures at the end of the efferent arteriole than did the normotensive animals. Nephron filtration rates were similar in both groups. At the same time, the hypertensive rats had higher nephron filtration fractions, and their glomerular blood flow was lower than that of the normotensive rats. These changes in the hypertensive animals were due to their higher afferent and efferent arteriolar resistances. Despite their high blood pressure, the glomerular ultrafiltration forces in hypertensive rats were almost identical with those in normotensive rats.

3. We conclude that the regulation of glomerular capillary pressure and filtration rate in young Kyoto hypertensive rats is due to a balance of afferent–efferent arteriolar tonus. The normal glomerular capillary pressures and low efferent arteriolar pressures of these animals are most likely the functional basis for the lack of structural changes at these sites. These results suggest that the Kyoto animal's renal response to hypertension resembles that of most essential hypertensive patients, both in function and morphology.

Key words: glomerular filtration, haemodynamics, renal arteriolar resistances, salt hypertension, spontaneous hypertension.

Abbreviations: GFR, glomerular filtration rate; GPF, glomerular capillary plasma flow.

Introduction
Genetic factors play an important role in the development of essential and experimental hypertension (Dahl, Heine & Tassinary, 1962; Okamoto & Aoki, 1963; Pickering, 1968). We recently found that genetically salt-sensitive rats develop hypertension, whereas salt-resistant rats remain normotensive after 3 months of high salt intake followed by 3–4 months of normal salt intake (Azar, Johnson, Hertel & Tobian, 1977; Azar, Johnson, Iwai, Bruno & Tobian, 1978). However, the chronic hypertensive state is accompanied by nephrosclerosis with decreased functional renal mass. The surviving nephrons of these kidneys failed to show an increase in afferent arteriolar resistance with hypertension, so that glomerular blood flow, filtration rate and capillary pressures all rose. We could not separate the effect of nephron loss from hypertension per se in these studies. We know that renal structure often remains basically unchanged in many essential hypertensive patients even after several years of high blood pressure (Sommers, Relman & Smithwick, 1958). In this aspect, the renal response to hypertension in the spontaneously hypertensive Kyoto rat resembles that of essential hypertensive patients. High blood pressure in these rats is primarily determined by genetic factors other than salt susceptibility (Louis,
Tabei & Spector, 1971), so we used them to study the effect of chronic hypertension on the regulation of glomerular capillary pressure and ultrafiltration.

Methods

We studied male rats of the Kyoto hypertensive and Kyoto normotensive control strains (A.R. Schmidt Co., Madison, Wisconsin, U.S.A.), kept on regular chow since weaning. When 15 weeks old, their blood pressures were measured without anaesthesia by the tail-cuff method (Friedman & Freed, 1949). The average of at least three readings was recorded. At this age, the hypertensive rats had an average systolic pressure of 164 mmHg (SE 4.0; n = 10); in normotensive animals this was 118 mmHg (SE 2.4; n = 9). Ten hypertensive and nine normotensive rats, weighing 300–370 g, were anaesthetized with Inactin [100 mg/kg (intraperitoneally) of the sodium salt of ethyl malonylthiourea; Promonta, Hamburg, Germany] and prepared for micropuncture (Azar, Tobian & Johnson, 1974). The following procedure kept blood pressure and packed cell volume constant in both hypertensive and normotensive rats. Immediately after anaesthesia, 1.5 ml of Krebs–Henseleit solution was injected into the femoral vein, followed by a constant infusion over 30 min (2.04 ml/h) of 50% fresh plasma (obtained from litter-mates) and 50% Krebs–Henseleit solution. [14C]Inulin was then added, to deliver 45 μCi/h. All solutions contained 15 units of heparin sodium/ml (Panheprin; Abbott Laboratories, Chicago, Illinois, U.S.A.). After equilibration for 1 h, studies were begun. At the end of each experiment the left kidney was removed, weighed, fixed in 10% phosphate-buffered formalin, embedded in paraffin and stained with haematoxylin and eosin and periodic acid–Schiff stains for light microscopy. Ten hypertensive and 12 normotensive kidneys were rated with a system in which coded sections were evaluated blindly for mesangial width, glomerular basement membrane, prominence of juxtaglomerular apparatus, thickness of arterial and arteriolar wall and intima, glomerular sclerosis, degenerative changes of proximal and distal tubules and interstitial width. At least two slides were evaluated for each animal and the tissues assigned to their appropriate groups after the ratings had been recorded. Total glomerular counts were performed in separate groups of 22 hypertensive and 18 normotensive kidneys by use of a sieving technique (Tryggvason & Kouvalainen, 1975).

Pressures, flows and resistances

Blood pressure was continuously monitored during micropuncture by a Statham strain gauge (model P23Db; Statham Instruments, Los Angeles, Calif., U.S.A.) and Beckman Dynograph recorder (model R611; Beckman Instruments, Fullerton, Calif., U.S.A.). Blood pressure was recorded at the time of micropressure measurement and at sampling of efferent arteriolar blood or tubular fluid. The mean of these values yielded the blood pressure for each animal. If an animal's blood pressure dropped by more than 15 mmHg during micropuncture, the experiment was ended and the data were not used in the study.

Each variable involved in single-nephron dynamics was based on a minimum of three determinations, which were then averaged and the resulting mean value was used for each rat. Proximal tubular fluid and plasma samples were collected simultaneously, then assayed for inulin concentrations to determine nephron glomerular filtration rate (nephron GFR) values (Azar et al., 1977). Glomerular capillary plasma flow (GPF) was calculated from nephron GFR and nephron filtration fraction; the latter was calculated from the simultaneously measured protein concentrations in femoral arterial and efferent arteriolar blood plasma as described by Bresler (1956). Procedures for collection and processing of efferent arteriolar blood have been reported (Azar et al., 1977). Differential pressure, as well as local rates of blood flow across afferent and efferent arterioles, were measured, so that resistances across these pre- and post-glomerular vessels could be estimated.

Hydrostatic pressures in tubules, ends of efferent arterioles (‘star vessels’) and small capillaries were measured with a servo-null micropipette transducer system (Azar et al., 1974). Glomerular capillary pressure was determined (Allison, Lipham & Gottschalk, 1972) as the sum of stop-flow pressure and the systemic colloid osmotic pressure. A valid stop-flow pressure assumes: (1) complete cessation of glomerular filtration, so that colloid osmotic pressure of glomerular capillary blood equals that of systemic arteries; (2) that glomerular capillary pressure remains unchanged due to either dilatation of the efferent, or constriction of the afferent, arteriole. This appears true in the normotenive rat, as well as in the Goldblatt hypertensive Munich rat (Azar et al., 1977). However, these assumptions may or may not be valid for the Kyoto hypertensive rat. Afferent arteriolar dilatation may be caused by cutting off flow to the
nephron’s macula densa during the stop-flow procedure. Glomerular capillary pressure would then change, causing values for nephron GFR measured in proximal tubules to exceed those measured in distal tubules (Schnermann, Wright, Davis, Stackelberg & Grill, 1970). To test this concept, we performed a separate experiment on seven Kyoto hypertensive and seven normotensive animals. Late proximal and distal convolutions of the same nephron were identified as before (Azar et al., 1978). Three tubular fluid samples of each kind were collected from each rat. The ratio of proximal to distal nephron GFR was 1.04 (SE 0.7; n = 7) in hypertensive rats and 1.01 (SE 0.05; n = 7) in normotensive animals, neither of which was significantly different from unity, suggesting that the interruption of flow to the macula densa did not significantly interfere with the nephron GFR and glomerular capillary pressure in either group.

Efferent arteriolar osmotic pressure (\(P_{\text{E}}\)) cannot be measured directly from the nanolitre-size samples collected. Therefore, usually, values for both efferent and afferent (\(P_{\text{A}}\)) osmotic pressures are calculated from measured afferent (\(C_{\text{A}}\)) and efferent (\(C_{\text{F}}\)) arteriolar protein concentrations, by using the Landis & Pappenheimer (1963) equation. However, because the albumin, rather than the globulin, concentration of a sample is primarily responsible for osmotic pressure, an increased globulin fraction may result in a falsely elevated calculation of osmotic pressure. Thus we use an IPM colloid osmometer (San Diego, California, U.S.A.) with a PM-30 membrane (Amicon Corp., Lexington, Mass., U.S.A.) to measure \(P_{\text{A}}\) directly. This value, together with the calculated nephron filtration fraction, was used to calculate \(C_{\text{A}}, C_{\text{F}}\) and \(P_{\text{E}}\) with a regression equation for osmotic pressures: \(P = 1.776c + 0.255c^2 - 0.001c^3\), where \(c\) = protein concentration in g/100 ml (Weller, Azar, Johnson & Azar, 1978).

**Analytical methods, calculations and statistics**

\(^{14}\text{C}\) radioactivity in tubular fluid, urine and plasma was counted with a Beckman liquid scintillation counter (Azar et al., 1977). Protein concentrations in efferent arteriolar and femoral arterial plasma samples were determined (Lowry, Rosebrough, Farr & Randall, 1951), at least in duplicate and usually in triplicate, with an Aminco fluoro-microphotometer (Silver Spring, Md., U.S.A.), as described by Brenner, Falchuk, Kelmowitz & Berliner (1969). For calculations, see the Appendix.

Values are given as mean ± SEM. Comparisons were made by unpaired t-tests, \(P < 0.05\) being considered significant (Snedecor & Cochran, 1967). Coefficient of variation was calculated in each rat with respect to pressures in the tubules (free and stop-flow) and star vessels, nephron GFR and nephron filtration fraction. This becomes critical because single-nephron function is derived from data obtained from several nephrons, thus requiring an homogeneous nephron population.

**Results**

Light-microscopic examination showed that hypertensive kidneys were indistinguishable from age-matched normotensive kidneys.

The average value of the electronically integrated blood pressure obtained during micropuncture in the two groups of rats is shown in Table 1. The body and kidney weight, total number of glomeruli per kidney and total GFR were similar in both groups. The packed cell volumes measured immediately after anaesthesia were similar in hypertensive and normotensive rats: 47.5% (SE 0.7; \(n = 10\)) and 47.1% (SE 0.6; \(n = 9\)) respectively. However, during micropuncture, packed cell volume was higher in the hypertensive than in the normotensive animals.

Single nephron results are shown in Table 1. Although glomerular capillary pressure was similar in the two groups, the pressure in the postglomerular vascular bed of the hypertensive rats was significantly lower. Nephron GFR was almost identical in both groups. Nephron filtration fraction in the hypertensive rats averaged 33% above that in the normotensive animals, so that the calculated glomerular blood flow of the former was reduced by 30% in comparison with that of the latter (\(P < 0.05\)).

Afferent arteriolar resistance was 159% higher in the hypertensive than in the normotensive rats (\(P < 0.001\)); efferent arteriolar resistance of the hypertensive animals was 55% above that of the normotensive rats (\(P < 0.001\)). Afferent arteriolar resistance comprised 75% of the total resistance to glomerular blood flow in the hypertensive (SE 1; \(n = 10\)) and 65% in the normotensive groups (SE 2; \(n = 9\)); \(P < 0.001\).

The unchanged glomerular transcapillary hydraulic pressure difference in hypertensive animals was due to their similar intratubular and glomerular capillary pressures. Both afferent and efferent arteriolar ultrafiltration pressures were unchanged; however, the high values observed at
the efferent end of the glomerulus indicate that filtration pressure equilibrium was not achieved in either group. The value of the glomerular capillary ultrafiltration coefficient ($K_c$) can be determined only when filtration pressure equilibrium is not reached (Deen, Robertson & Brenner, 1972). The average $K_c$ for the hypertensive group was 0.045 nl s$^{-1}$ mmHg$^{-1}$ (SE 0.003; $n = 10$), and that of the normotensive group was 0.051 nl s$^{-1}$ mmHg$^{-1}$ (SE 0.004; $n = 9$). At the same time, net ultrafiltration pressure was 18.3 mmHg (SE 1.0; $n = 10$) in the hypertensive and 17.5 mmHg (SE 1.3; $n = 9$) in the normotensive animals. These values were not statistically different.

**Discussion**

We found that hypertensive animals were difficult to work with under surgical conditions. Their exaggerated cardiovascular reaction to ‘psychological stress’ (Hallback, 1975) is also reflected in physiological variables under anaesthesia. For example, packed cell volume is progressively increased during the procedure. Infusion of crystalloid solutions at a rate that maintained the packed cell volume in normotensive rats resulted in an average 13% increase in these values for hypertensive animals. The prolonged stress of anaesthesia and surgery may have induced this change because blood pressure was constant and packed cell volume measured immediately after anaesthesia was similar in both groups. Genetic factors, age and/or duration of hypertension may also contribute to changes in packed cell volume. In previous studies of aged ‘post-salt’ hypertensive animals, the infusion of a crystalloid solution resulted in a packed cell volume equal to the normotensive control value (Azar et al., 1978). We used a crystalloid–colloid infusion in this study to maintain the naturally occurring similarity of packed cell volumes in hypertensive and control animals. It is possible that this solution introduced...
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a new variable into our study; however, we think that it actually restored ‘normality’ to the surgically stressed animals by eliminating the effect of elevated packed cell volume on renal vascular resistance (Myers, Deen, Robertson & Brenner, 1975).

The normal GFR seen in early essential hypertension (Goldring, Chasis, Ranges & Smith, 1941) is also found in Kyoto hypertensive rats. In these animals the calculated number of ‘functional’ nephrons (GFR/nephron GFR) was similar (34 784 glomeruli/kidney) to the number obtained by direct glomerular count, 33 837 (SE 686; n = 22), indicating that cortical nephron GFR is representative of the whole-kidney GFR.

It is known that the degree and extent of vascular damage correlates with the severity of sustained hypertension (Byrom, 1976). We expected no structural changes in the arteries and arterioles of the hypertensive kidneys because our rats were young, and the period of severe hypertension was short (Limas, Limas, Ragan & Freis, 1976). However, this does not preclude the possibility of altered collagen and non-collagen protein metabolism in the vasculature of these animals (Yamori, Nakada & Lovenberg, 1976). Efferent arteriolar damage is rarely seen in essential or experimental hypertension (Heptinstall, 1966). This morphological characteristic may be explained by the absence of hypertension in these vessels, as demonstrated in the present studies.

There was an elevation of both afferent and efferent arteriolar resistances in the Kyoto hypertensive rat. The 90% increase in aortic–glomerular capillary pressure difference observed in the hypertensive animal was accompanied by a 30% decrease in glomerular blood flow. The increased afferent arteriolar resistance reflects primarily the change in pressure difference, whereas the change in flow is related to the increase in efferent arteriolar resistance, since the pressure difference from glomerular capillary to efferent arteriole remained relatively unchanged.

Hypertensive patients have higher filtration fractions (Goldring et al., 1941), and this study provides insight into the pathophysiology of this increase, which appears to be the result of a fall in GPF, since the net glomerular transcapillary hydraulic pressure and nephrone GFR are unchanged. Lower GPF tends to increase net glomerular transcapillary osmotic pressure.

When filtration pressure disequilibrium exists, nephron GFR is determined by four factors: GPF, plasma osmotic pressure, net glomerular transcapillary hydraulic pressure and ultrafiltration coefficient (Deen, Robertson & Brenner, 1972). The last three factors were essentially unchanged in the hypertensive rats, whereas GPF dropped. Normally, a fall in GPF would cause a higher net glomerular transcapillary osmotic pressure and thus a lower nephron GFR. In our study, the decline in GPF in the hypertensive animals should have been enough to over-ride the net glomerular transcapillary hydraulic pressure and so reduce nephron GFR in proportion to the decrease in GPF. In this situation, nephron filtration fraction would have been lowered. However, efferent arteriolar resistance increased, thereby maintaining net glomerular transcapillary hydraulic pressure and nephron GFR, whereas GPF remained low and filtration fraction increased. Normal glomerular capillary pressure and nephron GFR in the structurally unaltered kidney is preserved by the balance of afferent and efferent arteriolar tone. The mechanism behind this regulation is unclear, but the increase in afferent arteriolar resistance could be attributed to an autoregulatory adjustment, which is more sensitive than normal (Lowenstein, Beranbau, Chasis & Baldwin, 1970).

Differences in afferent–efferent arteriolar regulation exist between the experimental states of Kyoto hypertension and salt hypertension, as demonstrated by the natural history and renal complications of hypertension in each. These regulatory differences might be viewed in the light of the primary role played by the kidney in salt hypertension (Dahl, Knudsen & Iwai, 1970). Normotensive salt-sensitive kidneys have fewer nephrons (Azar, Weller, Bruno, Livingston & Iwai, 1977), and renin activity is decreased under different stimulatory conditions (Iwai, Dahl & Knudsen, 1973); however, it is not known whether this is the reason for the decreased single nephron vascular resistance found in these animals (Azar et al., 1977). This dynamic characteristic is probably one cause of the accelerated renal damage seen once hypertension develops (Jaffe, Sutherland, Barber & Dahl, 1970), whereas, in the Kyoto hypertensive kidney, normal structure is maintained for many months. Even though glomerular filtration is maintained by a series of successive adaptive mechanisms long after the onset of hypertension, the Kyoto hypertensive kidney may still play a primary role in the pathogenesis of hypertension if its tubular salt regulation is defective. Finally, the answer concerning the role of the kidney in blood pressure regulation in the spontaneously hyper-
tensive rat must await studies conducted before the development of hypertension.

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APPENDIX

Calculations

Nephron glomerular filtration rate (NGFR): total $^{14}$C radioactivity counts of tubular fluid/(plasma $^{14}$C concentration $\times$ time of collection). The glomerular plasma flow (GPF) is calculated as

$$GPF = \frac{NGFR}{(1 - C_A/C_P)}$$

where $C_A$ is the systemic protein concentration, $C_P$ is the efferent peritubular capillary protein concentration and $(1 - C_A/C_P)$ is the nephron filtration fraction (NFF = NGFR/GPF). Glomerular blood flow (GBF) was determined from the relationship

$$GBF = \frac{GPF}{(1 - PCV)}$$

where PCV is the packed cell volume expressed as a fraction of 1. Afferent arteriolar resistance (RA) is defined as

$$RA = \frac{BP - PGC}{GBF}$$

where BP is the electronically integrated mean blood pressure and PGC is the glomerular capillary pressure (both in mmHg). Efferent arteriolar resistance (RE) is defined as

$$RE = \frac{(PGC - PE)}{(GBF - NGFR)}$$

where PE is efferent peritubular capillary hydrostatic pressure. Total arteriolar resistance (RT) = RA + RE. Mean net glomerular transcapillary hydraulic pressure

$$\Delta P = (PGC) - PT$$

where PT refers to proximal tubular pressure. Net driving force for ultrafiltration was determined as

$$PUF = \Delta P - \Delta \Pi$$

where $\Delta \Pi$ (IIGC − IIT) is the transmembrane oncotic pressure difference, IIGC and IIT represent the oncotic pressure at the glomerular capillaries and Bowman's capsular space respectively. IIT is only negligible, even in rats with heavy proteinuria; therefore it is omitted (Maddox, Bennett, Deen, Glasscock, Knutson, Daugharty & Brenner, 1975).

According to the Starling relationship, the rate of ultrafiltration per single glomerulus, NGFR, is given by the expression:

$$NGFR = KT(\Delta P - \Delta \Pi)$$

where $\Delta \Pi$ represents the transmembrane oncotic pressure difference, IIGC and IIT represent the oncotic pressure at the glomerular capillaries and Bowman's capsular space respectively. IIT is only negligible, even in rats with heavy proteinuria; therefore it is omitted (Maddox, Bennett, Deen, Glasscock, Knutson, Daugharty & Brenner, 1975).

The ultrafiltration coefficient (KF), the product of surface area (S) and effective hydraulic permeability (K) of the filtering capillaries, is calculated from the above equation and a differential equation which gives the rate of change of protein concentration with distance along an idealized glomerular capillary. Solution of this differential equation, described in detail by Deen et al. (1972) and Blantz (1974), allows calculation of IIGC.

References


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