Virchows Archiv A Pathol Anat (1989) 415:565–569

Virchows Archiv A
Pathological Anatomy and Histopathology

Variations in arterioles in spontaneously hypertensive rats

Morphometric analysis of afferent and efferent arterioles

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Summary. In the present study, the diameters of afferent and efferent arterioles of kidneys from spontaneously hypertensive rats (SHR) were evaluated and compared with those from Wistar Kyoto rats (WKY) using a vascular cast model. At 4 weeks of age, the blood pressure was slightly higher in SHR than in WKY (124 ± 1 vs 116 ± 7 mmHg, ns). The diameters of afferent arterioles in SHR were smaller than those in WKY (10.3 ± 0.6 vs 12.3 ± 0.7 μm, $P < 0.001$), whereas the diameters of efferent arterioles were comparable in the two strains. At 20 weeks of age, the blood pressure was markedly elevated in SHR than in WKY (192 ± 5 vs 140 ± 4 mmHg, $P < 0.001$). The diameters of afferent arterioles in SHR at this age were much smaller than those in WKY (14.3 ± 0.5 vs 17.1 ± 0.6 μm, $P < 0.01$). The diameters of efferent arterioles in SHR were, however, larger than those in WKY (15.4 ± 1.2 vs 12.9 ± 0.4 μm, $P < 0.05$). The net effect of these changes in arteriolar size helps to maintain normal intraglomerular pressure and to protect glomeruli from damage due to hypertension.

Key words: Vascular cast – Arteriole – Kidney – Spontaneously hypertensive rats

Introduction

Recent studies have shown that an increase in the intraglomerular hydraulic pressure plays an important role in the progression of glomerular diseases independent of the initial cause (Hostetter et al. 1981; Brenner 1985; Brenner et al. 1986). The intraglomerular hydraulic pressure is determined by transmission of the systemic blood pressure and also by the pre- and post-glomerular vascular resistance. A rise in the pre-glomerular resistance or a fall in the post-glomerular resistance reduces the intraglomerular hydraulic pressure. Conversely, a fall in the pre-glomerular resistance or a rise in the post-glomerular resistance raises it (Brenner et al. 1986). Morphological assessment of vascular resistance can be achieved by measuring the diameters of resistance vessels (Wilson 1986).

The purpose of the present work was thus to quantitatively evaluate the state of the renal arterioles in spontaneously hypertensive rats (SHR) by employing a vascular casting method and thereby obtain a more complete understanding of the pathophysiology of renal vasculature in the development of hypertension.

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Materials and methods

SHR of 4 and 20 weeks old and their normotensive control Wistar Kyoto rats (WKY) of the same ages were used. Each group consisted of five rats. They were fed on normal rat pellets and tap water was accessible ad libitum. Renal vascular casts were obtained following the method of Gattone et al. (1983; Gattone and Evan 1986).

Systolic blood pressure was measured in conscious rats by the tail cuff method just before the experiment. Under intraperitoneal pentobarbital anesthesia, a polyethylene catheter PE-60 was inserted retrogradely into the abdominal aorta with the tip of the catheter placed just below the left renal artery. The systemic blood pressure was monitored via the catheter using a three way stopcock. Immediately after the proximal aorta was ligated between the right and left renal arteries and the left renal vein was opened by a small incision for outflow, 0.9% saline was infused at room temperature for a few s, followed by 2.5% glutaraldehyde in 0.1 M phosphate buffer at pH 7.4 for 3 min to fix the left kidney. To ensure fixation of the vasculature in the functional state, infusion was performed by a hand syringe and the infusion pressure was adjusted to be the same as the mean arterial pressure measured just before the ligation of the proximal aorta (Gattone et al. 1983). After fixation, the acryl resin (Mercox, Dai-Nihon Inki, Tokyo, Japan) was infused for the preparation of a cast of the vascular system in the left kidney.

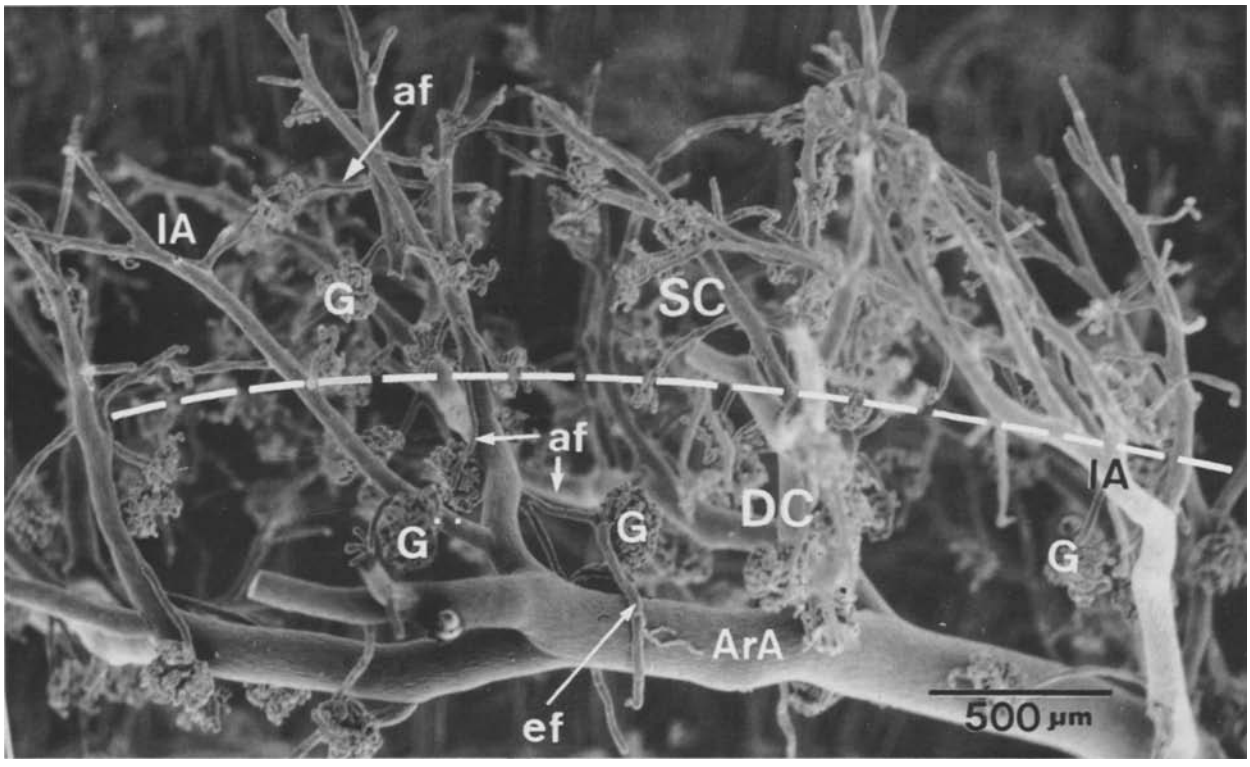

Fig. 1. Low magnification scanning electron micrograph of renal vascular cast. The vasculature from the arcuate artery (ArA) through the interlobular artery (IA), afferent arteriole (af) and glomeruli (G) can be easily identified. SC, superficial cortex; DC, deep cortex. (× 38)

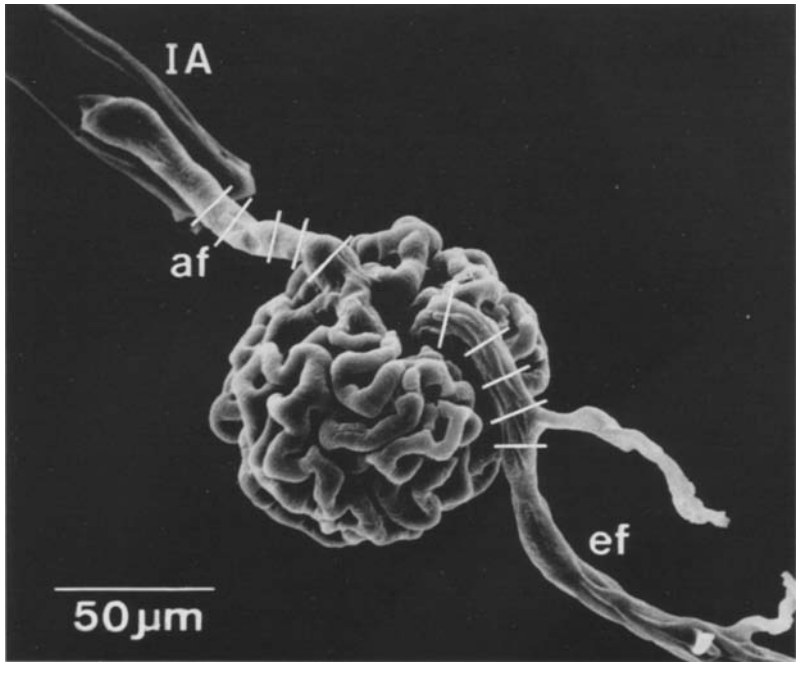

Fig. 2. Typical cast of vasculature from the afferent arteriole (af) through the glomerulus (G) and efferent arteriole (ef). Diameters of arterioles were measured at five points in the segment 50 µm in length from glomerulus on photographic prints. A mean value of five diameters measured was taken as the diameter of the arteriole concerned. (× 430)

A portion of the renal tissue was examined by light microscopy. The rest of the tissue was incubated at room temperature for 24 h so that the vascular casts cured sufficiently. The renal tissue was digested and removed in 30% sodium hypochlorite solution. The casts were rinsed several times with distilled water and then air dried.

The glomerular casts were subsequently dissected under a stereomicroscope. Only glomerular casts with both afferent

and efferent arteriolar casts were chosen for this study. They were mounted on stubs for scanning electron microscopy and transferred to a sputter coater for coating with gold palladium for 90 s at 1.0 mvolts. Hitachi S-650 scanning electron microscope (SEM) (Hitachi, Japan) (SEM) was used for examining and photographing the casts.

Casts of afferent and efferent arterioles were examined in the SEM at 600×, using an accelerating voltage of 20 kV and a working distance of 15 mm. Five to ten glomeruli for each superficial cortex and the same number of glomeruli from each deep cortex in one kidney were examined (Fig. 1). Diameters of arterioles were estimated on the photographic prints. A diameter was measured as a distance between parallel edges of the selected arteriole by drawing lines perpendicular to arteriolar lateral lines (Fig. 2). In a segment of arteriole 50 μm from the glomerulus, diameters were measured at five points and a mean vessel diameter was determined by averaging all measurements. The data were pooled in each group for 50 to 100 glomeruli and analyzed by one-way analysis of variance. *P*-value equal to or less than 0.05 was considered significant.

Results

The blood pressure of 4-week-old SHR was slightly higher than that of the WKY of the same age (124 ± 1 vs 116 ± 7 mmHg, ns). The blood pressure of 20-week-old SHR was significantly higher than that of WKY of the same age (192 ± 5 vs 140 ± 4 mmHg, $P<0.001$). There was no significant difference in the body weight between the two strains at either age (Fig. 3).

On light microscopic examination of the renal tissue with haematoxylin-eosin staining, no remarkable glomerular damage was found in SHR. There was only slightly enhanced hyalinosis and sclerosis compared with WKY at 20 weeks of age.

As arterioles from both superficial and deep cortexes showed the same general pattern, the data from the whole cortex are shown. At 4 weeks of age, the diameters of afferent arterioles in SHR

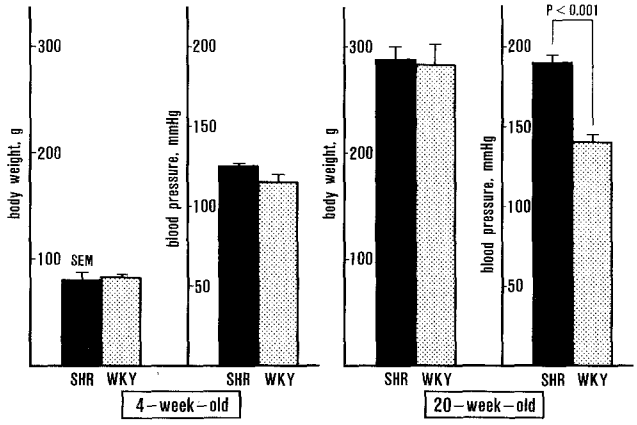

Fig. 3. There was no significant difference in body weight either at 4 weeks or at 20 weeks. At 4 weeks blood pressure in SHR is slightly but not significantly higher than in WKY. At 20 weeks it was significantly higher in SHR than in WKY ($P<0.001$)

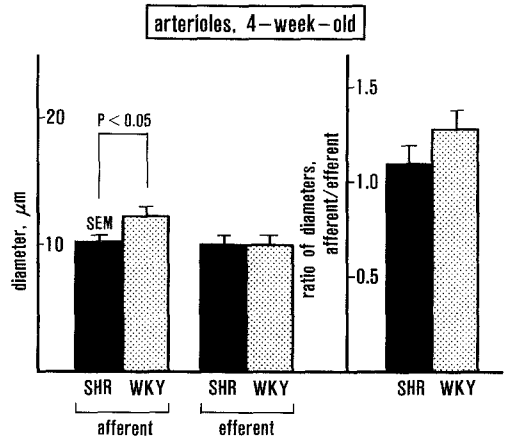

Fig. 4. Diameters of renal arterioles in vascular casts from 4-week-old rats. Diameters of afferent arterioles were smaller in SHR than in WKY ($P<0.05$). The ratio of afferent to efferent arteriolar diameters in SHR was slightly smaller but it was not significant

were slightly but significantly smaller than those in WKY (10.3 ± 0.6 vs 12.3 ± 0.7 μm, $P<0.05$) whereas the diameters of efferent arterioles showed no significant difference between the two strains. The ratio of the diameters of afferent arterioles to those of efferent arterioles was comparable in the two groups (1.1 ± 0.1 vs 1.2 ± 0.1, ns) (Fig. 4). Representative casts from SHR and WKY at 20 weeks of age are shown in Fig. 5. At this age the diameters of afferent arterioles in SHR were significantly smaller than in WKY (14.3 ± 0.5 vs 17.1 ± 0.6 μm, $P<0.01$) and, on the other hand, the diameters of efferent arterioles in SHR were significantly greater than in WKY (15.4 ± 1.2 vs 12.9 ± 0.4 μm, $P<0.05$). The ratio of the diameters of afferent arterioles to that of efferent arterioles were significantly smaller in SHR than in WKY (1.0 ± 0.1 vs 1.3 ± 0.1, $P<0.001$) (Fig. 6).

Discussion

Direct observation of renal microvasculature facilitates the understanding of renal pathophysiological conditions, as the microvasculature play an important role in the regulation of renal function (Gattone and Evan 1986). Through controlled vascular perfusion fixation and renal vascular casting, the microvasculature can be fixed in its functional state and thus direct observation and quantitative analysis are possible (Gattone et al. 1983). Recently, several studies on the changes in arterioles in SHR and in other renal diseases were reported utilizing vascular casting (Gattone et al. 1983; Gattone and Evan 1986). However, only afferent arterioles have been thus far evaluated and there is little available information regarding efferent arterioles.

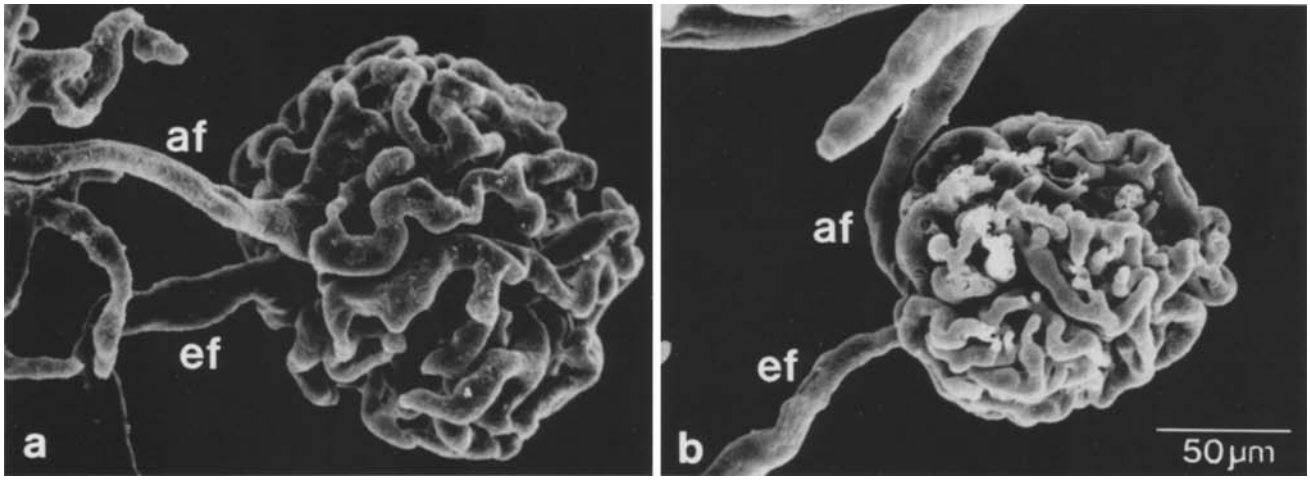

Fig. 5. Vascular casts of arterioles from 20-week-old rats. (**a**) SHR, (**b**) WKY. An afferent arteriole in SHR (af in **a**) is significantly smaller than that in WKY (af in **b**) and an efferent arteriole in SHR (ef in **a**) is significantly larger than that in WKY (ef in **b**). (×460)

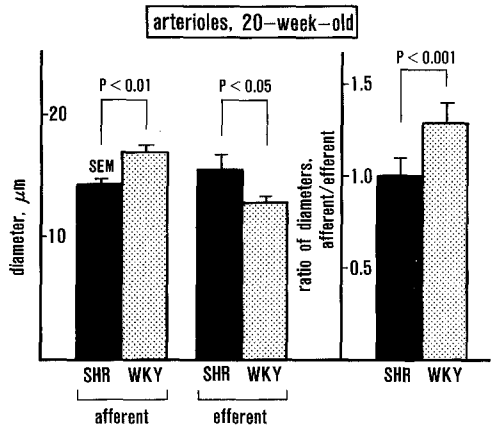

Fig. 6. Diameters of renal arterioles in vascular casts from 20-week-old rats. Diameters of afferent arterioles were smaller in SHR than in WKY ($P<0.01$). And diameters of efferent arterioles were larger in SHR than those in WKY ($P<0.05$). Therefore, the ratio of afferent to efferent arteriolar diameters was much smaller in SHR than in WKY ($P<0.001$)

Recent micropuncture studies have suggested that the elevated intraglomerular hydraulic pressure is one of the most important factors in the progression of renal diseases (Hostetter et al. 1981). As the intraglomerular pressure is determined by the systemic pressure as well as by the resistance of both afferent and efferent arterioles (Brenner et al. 1986), both afferent and efferent arterioles must be evaluated simultaneously in order to understand the effects of high blood pressure on glomeruli. Thus, in the present study, we selected glomerular casts with both afferent and efferent arterioles (Fig. 2), measured the diameters and calculated the ratios. The ratios of diameters of afferent to efferent arterioles is one of the important variables in determining the intraglomerular pressure (Dilley et al. 1984). When these values are elevated, the intraglomerular hydraulic pressure rises and vice versa, assuming that the systemic pressure is constant.

The results of the present study showed that afferent arterioles are constricted and efferent arterioles are dilated in 20-week-old SHR in a persistent hypertensive state compared with WKY ($P<0.01$ and $P<0.05$, respectively, Fig. 6). The ratio of the diameters was lower in SHR than in WKY ($P<0.001$). The net effect of these arteriolar changes may contribute to the maintenance of normal intraglomerular hydraulic pressure in spite of systemic high blood pressure. These morphological findings were consistent with results from haemodynamic studies that the renal afferent arteriolar resistance is elevated and intraglomerular pressure is normal in SHR (Bank et al. 1983; Dilley et al. 1984). In the so-called prehypertensive state of the 4-week-old rats, afferent arterioles in SHR was constricted ($P<0.05$) but efferent arterioles showed no change (Fig. 4). The ratio of the diameters was comparable in both strains. In this stage, blood pressure was already slightly higher in SHR than in WKY, though the difference was not statistically significant in the present study (Fig. 3).

The results concerning afferent arterioles in the present study were consistent with those previously reported by other researchers who applied the same method (Gattone et al. 1983; Gattone and Evan 1986). Smaller arterioles in SHR have been confirmed by the microspheric method (Hsu and Slavicek 1982). Gattone et al. (1984) showed that denervation did not abolish this strain-related dif-

ference and suggested that this change was possibly due to congenital hypoplasia. In the present study, efferent arteriolar dilatation and a smaller ratio of diameters were shown in SHR at an established hypertensive stage (20-week-old, Fig. 6); these were not found in the prehypertensive stage (4-week-old, Fig. 4). Therefore, both afferent and efferent arterioles changed with the development of systemic hypertension and seemed to protect glomeruli from hypertension-induced damage. Another explanation for the arteriolar changes seen in SHR is a reaction secondary to hypertension but not a congenital abnormalities, although the precise mechanisms of these arteriolar changes remains to be clarified and defined.

These arteriolar changes might explain the rather slight glomerular lesions in SHR in spite of the high systemic blood pressure (Dworkin et al. 1986). In contrast, in deoxycorticosterone (DOCA)-salt hypertensive rats, the development of elevated intraglomerular pressure and severe glomerular damage were reported (Dworkin et al. 1984). Arteriolar changes might be different in various types of hypertension and these differences might explain the variation in glomerular damage. In this context, morphometric evaluation of renal arterioles will help us to obtain a more complete understanding of the pathophysiology of hypertension and to see the relationship between arteriolar states and hypertension-induced glomerular damage.

Acknowledgments. The authors express their tanks to Mrs. Sanae Ogawa for her skillful technical assistance.

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Received April 28, 1989 / Accepted June 20, 1989