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Variations in renal arteriolar diameter in deoxycorticosterone acetate-salt hypertensive rats

A microvascular cast study

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Summary. The relation between hypertensive glomerular damage and arteriolar diameter was examined in a microvascular cast study in deoxycorticosterone acetate (DOCA)-salt hypertensive rats. The blood pressure and urinary protein excretion increased progressively in the DOCA rats. In controls afferent arteriolar diameters increased during the course of the experiment, and efferent arteriolar diameters remained unchanged. In the DOCA rats, however, afferent arteriolar diameters did not change significantly, while efferent arteriolar diameters increased. Histological studies showed severe arteriolosclerosis and glomerulosclerosis in the DOCA rats. The results show that these arteriolar changes might contribute to the reduction of glomerular capillary pressure in the development of DOCA-salt hypertension. However, they are not sufficient to protect glomeruli from hypertensive damage.

Key words: Vascular cast – Arteriole – Kidney – DOCA-salt hypertensive rats

Introduction

Intraglomerular hypertension plays a possible role in the pathogenesis of glomerular injury (Azar et al. 1977; Hostetter et al. 1981; Brenner et al. 1982). Micropuncture studies have demonstrated that glomerular capillary pressure is significantly increased in deoxycorticosterone acetate (DOCA)-salt hypertensive rats (Dworkin et al. 1984). However, spontaneously hypertensive rats (SHR) are reported to show normal glomerular capillary pressure in spite of systemic hypertension (Dilley et al. 1984). A previous microvascular cast study showed that afferent arterioles are constricted and efferent arterioles dilated in SHR (Kimura et al. 1989). These arteriolar changes may help to maintain a normal glomerular capillary pressure and protect glomeruli from hypertensive damage, explaining the minimal glomerular sclerosis in SHR despite severe systolic hypertension (Kimura et al. 1989). In DOCA-salt hypertensive rats, however, glomerular damage is seen at an early stage of hypertension and progresses (Hewitson et al. 1988).

In the present study we examined variations in renal arteriolar diameters in DOCA-salt hypertensive rats in order to examine the relationship between hypertensive glomerular damage and arteriolar diameters.

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Materials and methods

Twelve adult male Wistar rats weighing 250–300 g underwent right nephrectomy under ether anaesthesia. After 1 week of recovery, rats received weekly subcutaneous injections of DOCA (30 mg/kg body weight) in sesame oil and were given 1% saline for drinking water. Twelve male Wistar rats of the same age were used as controls. The control rats were fed standard chow and tap-water. Experiments were performed in the 2nd week of the DOCA-salt treatment. Blood pressure was measured in unanaesthetized rats by the tail-cuff method. Then the rats were placed in individual metabolic cages and 24-h urine was collected for measurement of urinary protein and *N*-acetyl-β-D-glucosaminidase (NAG) excretion. Subsequently, six rats from each group were used in the microvascular study. The same procedure was repeated at the 7th week using the remaining six rats from each group.

The microvascular cast study was performed following the method previously reported (Kimura et al. 1989). A polyethylene catheter was inserted into the abdominal aorta and perfusion fixation of the left kidney was performed with 2.5% glutaraldehyde in 0.1 M phosphate buffer at pH 7.4 for 3 min at the same pressure as the mean arterial pressure of each rat. After the fixation, the acryl resin (Mercox, Dai-Nihon Inki, Tokyo, Japan) was infused for the preparation of microvascular casts. A portion of the renal tissue was fixed in 95% ethanol for light microscopic examination. The rest of the renal tissue was digested in 30% sodium hypochlorite solution and casts were obtained. These were examined in a scanning electron microscope (SEM) (Hitachi S-450, Japan) at ×600 magnification. Fifteen to 20 glomeruli from the outer cortex and the same number of glomeruli from the inner cortex of one kidney of each rat were examined. On the photographic prints, the arteriolar diameters were measured at five points in 50-μm arteriolar segments from the vascular pole of each glomerulus. A mean arteriolar diameter was determined by averaging the five

diameters measured. The data were pooled in the outer or inner cortex of each group for 90–120 glomeruli and compared among groups.

In light microscopy, glomerular and arteriolar damage was scored following the method of Kimura et al. (1990) and Hewitson et al. (1988). Glomerular sclerosis is scored as S0 for a normal glomerulus; S1, mild sclerosis (less than 30%); S2, moderate segmental sclerosis (30%–60%); S3, severe segmental sclerosis (more than 60%); S4, global sclerosis. Arteriolar damage is categorized as A0, no abnormality; A1, slight thickening of media; A2, hyalinosis in focal area together with moderate thickening of media; A3, medial thickening with global hyalinosis; A4, fibrinoid necrosis and/or cellular hyperplasia with narrowing of arteriolar lumen and/or thrombus formation. The degree of each glomerular and arteriolar damage was expressed as follows: glomerulosclerosis (or arteriolosclerosis) score = [0 × number of S0 (or A0) + 1 × number of S1 (or A1) + 2 × number of S2 (or A2) + 3 × number of S3 (or A3) + 4 × number of S4 (or A4)]/[number of S0 (or A0) + S1 (or A1) + S2 (or A2) + S3 (or A3) + S4 (or A4)].

The data are expressed as means ± SEM. Statistical differences between the means were analysed by one-way analysis of variance. Differences were considered to be significant at a P level below 0.05.

Results

The blood pressure of DOCA-salt hypertensive rats was already significantly higher than that of the control Wistar rats at the 2nd week (113 ± 4 in the controls vs 144 ± 5 mmHg in DOCA-salt rats, $P < 0.001$). At the 7th week, the blood pressure of DOCA-salt rats increased further, whereas that of the control did not change (116 ± 4 vs 172 ± 8 mmHg, $P < 0.001$) (Fig. 1). The urinary excretion of protein and NAG significantly increased in DOCA-salt rats (Table 1).

At the 2nd week, the diameters of afferent arterioles in both the outer and inner cortexes showed no significant difference between the controls and the DOCA-salt rats (outer cortex: 13.5 ± 0.6 in the controls vs 13.6 ± 0.3 μm in DOCA-salt rats, NS; inner cortex: 13.6 ± 0.5 vs 13.8 ± 0.3 μm, NS), the diameters of efferent arterioles in both cortices were slightly, but not significantly, larger in the DOCA-salt rats than in the controls (outer cortex: 13.3 ± 1.2 vs 13.9 ± 0.4 μm, NS; inner cortex: 15.0 ± 0.9 vs 16.4 ± 0.6 μm, NS) (Fig. 2).

There were regional differences between the outer and inner cortices; the diameter of afferent arterioles showed no differences between both cortices, but those of efferent arterioles were larger in the inner cortex than in the outer cortex, in both the controls and the DOCA-salt rats.

Representative casts in both groups at the 7th week are shown in Fig. 3. At the seventh week, the diameters of afferent arterioles in the DOCA-salt rats were significantly smaller than those in the controls in both cortices (outer cortex: 15.7 ± 0.3 in the controls vs 13.3 ± 0.3 μm in DOCA-salt rats, $P < 0.01$; inner cortex: 16.0 ± 0.5 vs 14.2 ± 0.3 μm, $P < 0.01$). However, the diameters of efferent arterioles in the DOCA-salt rats were larger than those in the controls in the outer cortex (13.6 ± 0.4 vs

Table 1. Urinary protein and N-acetyl-β-D-glucosaminidase (NAG) excretion

		Protein (mg/day)	NAG (mU/day)
Control	2nd week	17 ± 2	15 ± 2.3
	7th week	20 ± 1	98 ± 47
DOCA-salt	2nd week	43 ± 22	154 ± 69*
	7th week	148 ± 2**	576 ± 153*

Values are given as mean ± SEM
* $P < 0.05$, ** $P < 0.01$ vs control at the same week

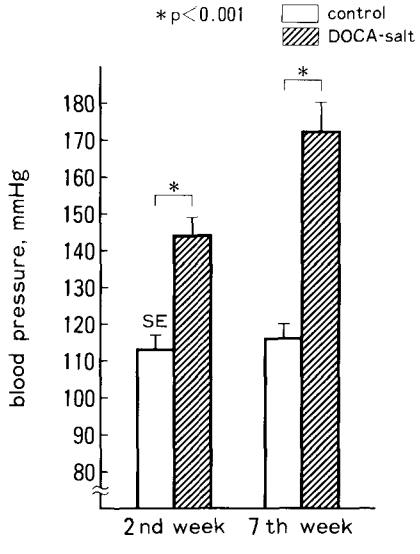

Fig. 1. Blood pressure. The blood pressures in the DOCA-salt hypertensive rats are significantly higher than those in the controls by the 2nd week and increase further more by the 7th week

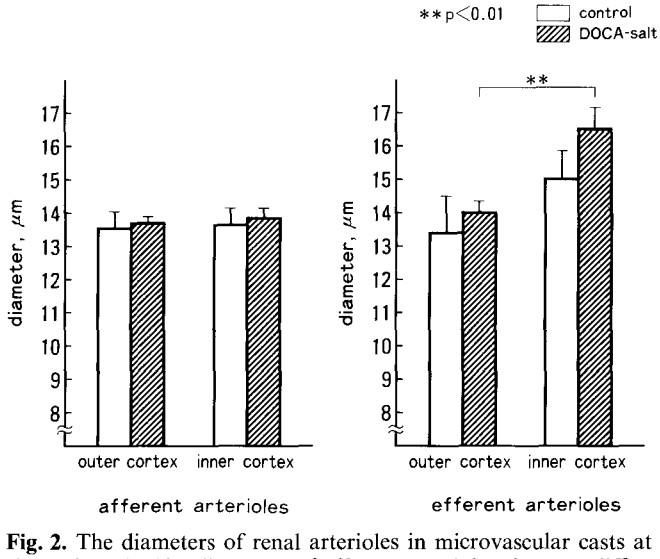

Fig. 2. The diameters of renal arterioles in microvascular casts at the 2nd week. The diameters of afferent arterioles show no difference between the controls and the DOCA-salt rats in either the outer or inner cortex. The diameters of efferent arterioles are slightly larger in the DOCA-salt rats than in the controls, in both cortices. The efferent arterioles in the inner cortex are larger than in the outer cortex in both the controls and the DOCA-salt rats ($P < 0.01$ in DOCA-salt)

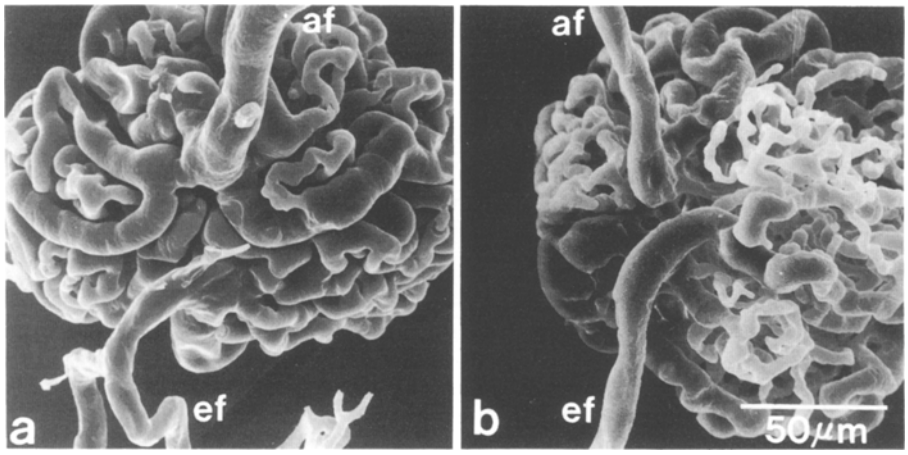

Fig. 3a, b. Representative microvascular casts of arterioles in the outer cortex at the 7th week. **a** Control; **b** DOCA-salt rat. An afferent arteriole in the DOCA-salt rat is smaller than that in the control and an efferent arteriole in the DOCA-salt rats is larger than in the control. *af*, Afferent arteriole; *ef*, efferent arteriole. ×600

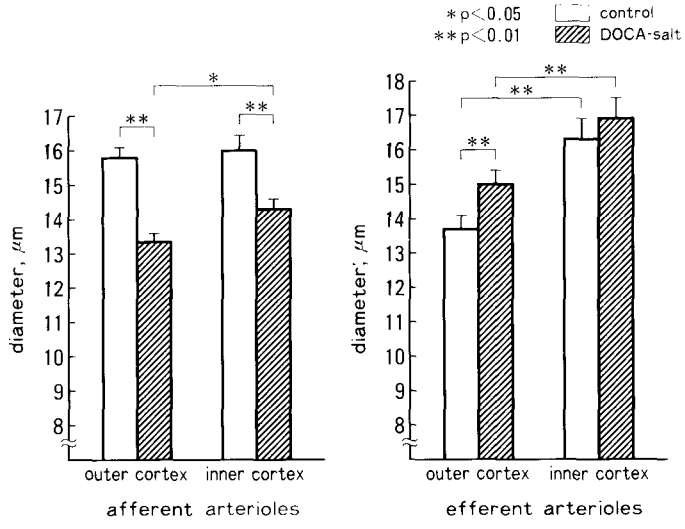

Fig. 4. The diameters of renal arterioles in microvascular casts at the 7th week. The diameters of afferent arterioles are significantly smaller in the DOCA-salt rats than in the controls ($P<0.01$). The diameters of efferent arterioles are larger in the DOCA-salt rats than in the controls. The efferent arterioles in the inner cortex are significantly larger than in the outer cortex both in the controls and in the DOCA-salt rats ($P<0.01$)

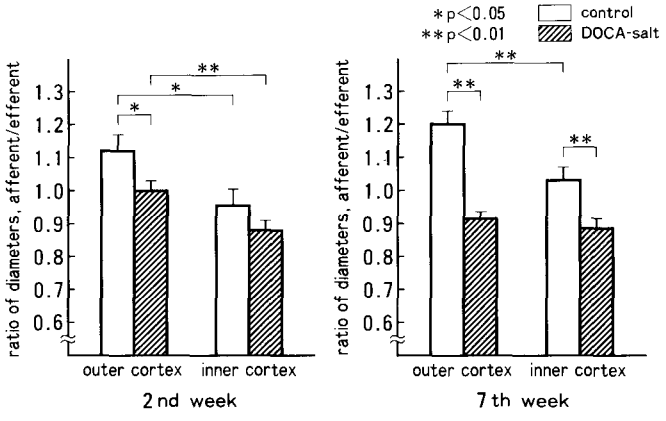

Fig. 5. The ratio of afferent to efferent arteriolar diameters. The ratio is smaller in the DOCA-salt rats than in the controls already at the 2nd week and more significantly decreased at the 7th week ($P<0.01$), especially in the outer cortex

15.0 ± 0.4 μm, $P<0.01$) and tended to be larger in the inner cortex (16.2 ± 0.6 vs 16.8 ± 0.6 μm, NS).

The diameters of afferent arterioles were significantly smaller in the outer cortex than in the inner cortex in DOCA-salt rats ($P<0.05$), whereas those in the controls showed no difference. The diameters of efferent arterioles were significantly larger in the inner cortex than in the outer cortex both in the controls and in the DOCA-salt rats ($P<0.01$) (Fig. 4).

The ratios of the afferent arteriolar diameters to the efferent arteriolar diameters (a/e ratios) are shown in Fig. 5). The a/e ratios were significantly smaller in the DOCA-salt rats than in the controls at the 2nd week in the outer cortex (1.11 ± 0.5 in the controls vs 1.00 ± 0.03 in DOCA-salt rats, $P<0.05$) and tended to be smaller in the inner cortex (0.95 ± 0.05 vs 0.88 ± 0.03, NS). At the 7th week, the a/e ratios in DOCA-salt rats were significantly smaller than those in the controls in both cortices (outer cortex: 1.20 ± 0.04 vs 0.91 ± 0.02, $P<0.01$; inner cortex: 1.03 ± 0.04 vs 0.88 ± 0.03, $P<0.01$).

Concerning regional differences, the ratios of both groups were significantly smaller in the inner cortex than in the outer cortex at 2nd week. However, at the 7th week, no regional difference was observed in DOCA-salt rats, while the ratios in the controls were significantly smaller in the inner cortex than in the outer cortex (Fig. 5).

On light microscopy, the DOCA-salt rats showed various grades of glomerulosclerosis with mesangial cell proliferation, tubular atrophy and lymphocytic infiltration in the interstitium. Arterioles showed marked medial thickening and hyalinosis. The scores of glomerulosclerosis and arteriolosclerosis in DOCA-salt rats tended to be greater than those in the controls by the 2nd week in both cortices, and were significantly greater at the 7th week.

The scores for glomerulosclerosis in the inner cortex were slightly, but not significantly, greater than those in the outer cortex both in DOCA-salt rats and in controls. However, the scores for arteriolosclerosis in the

Table 2. Scores of glomerulosclerosis and arteriolosclerosis

		Glomerulosclerosis		Arteriolosclerosis	
		Outer cortex	Inner cortex	Outer cortex	Inner cortex
Controls	2nd week	62±31	75±18	0±0	14± 4[3]
	7th week	50±10	64±16	5±3	17± 6
DOCA-salt rats	2nd week	126±17[1]	134±23	5±4	46±20[3]
	7th week	162±22[2]	181±19[2]	23±6[1]	81±11[2, 4]

Values are given as mean±SEM
[1] $P<0.05$; [2] $P<0.01$ vs. control at the same week; [3] $P<0.05$; [4] $P<0.01$ vs. outer cortex in the same group

inner cortex were significantly greater than those in the outer cortex in DOCA-salt rats (Table 2).

Discussion

We measured renal arteriolar diameters directly by using vascular casts and have found that afferent arterioles were constricted and efferent arterioles were dilated in DOCA-salt hypertensive rats. The diameter ratio in the DOCA-salt rats tended to decrease in the earliest stages of hypertension and was significantly decreased at the late stage. These changes in arterioles seem to be a factor in the reduction of the intraglomerular pressure, protecting them from the effects of systemic hypertension.

The arteriolar changes were consistent with the results of micropuncture studies which have shown that the afferent arteriolar vascular resistance is increased and the efferent arteriolar resistance is decreased in DOCA-salt rats (Dworkin et al. 1984). Furthermore, the afferent arteriolar constriction found is consistent with the results of a serial histological cross-sectional study, which showed that the lumen radius of the renal arteriole was decreased in DOCA-salt hypertension (Vial et al. 1989).

Using microvascular casts, Kimura et al. (1989) reported afferent arteriolar constriction and efferent arteriolar dilatation in SHR and suggested that these arteriolar changes might protect glomeruli from hypertensive damage. The same arteriolar changes were observed in the present study in DOCA-salt rats. Myogenic autoregulation of the glomerular filtration rate and tubuloglomerular feedback are possible mechanisms for these afferent arteriolar changes (Thurau 1964; Dilley and Arendshorst 1984; Moore and Mason 1986; Sanchez-Ferrer et al. 1989). The efferent arteriolar dilatation could be explained by the fact that efferent arteriolar resistance tended to decrease slightly with elevation of systemic pressure in normal Wistar rats (Robertson et al. 1972). Further studies are necessary to confirm the mechanism of arteriolar regulation.

On light microscopic examination, glomerulosclerosis was observed in the early stage of hypertension in the DOCA-salt rats. Glomerulosclerosis was observed to the same extent in the outer cortex and in the inner cortex. These light microscopic observations and arteriolar diameter changes suggest that arteriolar changes are not sufficient to protect glomeruli from the pressure damages in DOCA-salt rats. This assumption is consistent with the results of micropuncture studies in DOCA-salt rats (Dworkin et al. 1984) and in contrast, in SHR, glomerulosclerosis is mild (Kimura et al. 1989). Here, the glomerular capillary pressure is said to be normal (Dilley et al. 1984) and the arteriolar changes are assumed to be sufficient to protect glomeruli from pressure damage. The reason for enhanced glomerulosclerosis in DOCA-salt rats is not clear at present. The increased circulating blood volume observed in DOCA-salt rats (Villamil et al. 1982) might contribute to glomerulosclerosis through the increase in the intraglomerular flow and pressure (Brenner et al. 1972). Further studies are necessary to clarify this mechanism.

Arteriolosclerosis was more severe in the DOCA-salt rats than in the controls, and occurred primarily in the inner cortex in both groups. This may indicate that arteriolar pressure is higher in the inner than in the outer cortex and a higher perfusion pressure in the inner cortex might be due to the fact that efferent arteriolar diameters are larger in the inner cortex than in the outer cortex both in DOCA-salt rats and in the controls (Robertson et al. 1972).

In conclusions, we have examined the diameters of renal arterioles directly and have shown that afferent arterioles were constricted and efferent arterioles dilated in DOCA-salt hypertension. These changes may be a regulatory mechanism operating to reduce the intraglomerular pressure; however, they are not sufficient to protect glomeruli from hypertensive damage.

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