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Effects of Endothelin and Angiotensin II on Renal Hemodynamics in Experimental Mesangial Proliferative Nephritis

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Key Words
Glomerulonephritis
Endothelin
Angiotensin II
Hemodynamics
Hypertension

Abstract
The renal hemodynamic response to pressor substances in the diseased kidney has been suggested to be different from that in the normal kidney. The aim of this study was to investigate the effects of endothelin and angiotensin II on renal hemodynamics in experimental nephritis induced by the administration of antithymocyte serum in Wistar rats. This model showed mesangiolytic lesions in the glomeruli on day 2 and hypercellular lesions on day 8. Prior to the injection of either endothelin or angotensin II, the glomerular filtration rate and renal plasma flow were significantly lower in model rats on day 2 or day 8 than in the control rats. The basal glomerular filtration rate and renal plasma flow on day 8 were negatively correlated with the mesangium cell number. The injection of endothelin (0.5 ng/kg BW) led to a decrease in both renal plasma flow and glomerular filtration rate in rats on day 8 which was significantly greater than that in the control rats. Similarly, angiotensin II infusion (0.2 μg/kg BW) reduced both renal plasma flow and glomerular filtration rate in the rats on day 8 and the reductions were significantly greater than those in the control rats. In conclusion, renal hemodynamics in rats with mesangial proliferation of the kidney were more sensitive to both endothelin and angiotensin II than those in the normal kidney.

Introduction

Hypertension frequently coexists with glomerulonephritis and is one of the factors accelerating renal dysfunction [1]. Systemic hypertension per se seems to have little hemodynamic effect on the normal kidney in a chronic hypertensive state, because renal vasculatures autoregulate intraglomerular pressure and flow against the changes of systemic arterial pressure by regulating arteriolar resistance [2]. On the other hand, the inability of the renal vasculatures in the diseased kidney to autoregulate intraglomerular pressure has been suggested to lead to renal hyperhemodynamics [3]. Thus, the vascular response to the pressor substances in the diseased kidneys may be different from that in the normal kidneys. Endothelin (ET), which was recently disclosed, is a prominent and long-acting vasoconstrictor [4]. In others, angiotensin II (AII), which is one of the classical pressor substances, regulates renal circulation while reduc-

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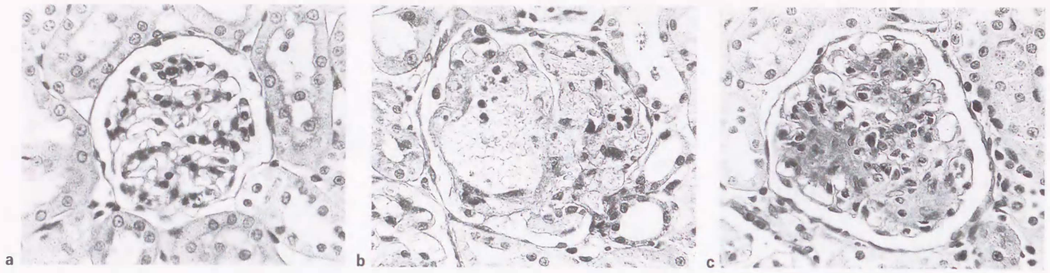

Fig. 1. **a** Normal glomerulus of control rat. PAS. **b** Glomerulus on day 2 after ATS injection showing a decrease in glomerular cells with mesangiolysis. **c** Glomerulus on day 8 showing hypercellularity.

ing the glomerular ultrafiltration coefficient [5]. Both ET and AII induce the contractile response of renal arterioles or mesangial cells [6, 7]. In this report, we have shown the effect of vasoconstrictors such as ET and AII on renal hemodynamics in the kidney with mesangial proliferation, using a rat model of glomerulonephritis induced by specific immunologic injury to the mesangial cells [8].

Methods

Induction of Experimental Glomerulonephritis

Antirat thymocyte serum (ATS) was produced by immunizing New Zealand White rabbits with 1 × 109 Wistar rat thymocytes in complete Freund's adjuvant, followed with 1 × 107 thymocytes given intravenously 2 and 4 weeks later [9]. Preimmunization serum was collected from the same animal and used in the control experiments as normal rabbit serum. Glomerulonephritis was induced in Wistar rats (150–200 g BW) by the intravenous administration of 1 ml ATS/100 g BW. The control animals received the same dose of normal rabbit serum instead of ATS. The rats were killed for a histological examination of the kidney tissue after the studies of inulin and *p*-aminohippurate clearances either on day 2 or 8 after ATS administration.

*Inulin and p-*Aminohippurate Clearances

Thirty-five rats were separated into two groups for the experiments of ET (N = 18) injection and AII (n = 17) infusion. Furthermore, rats were divided into three groups, consisting of the control rats and those examined on day 2 and on day 8 after ATS injection. The rats were anesthetized with sodium pentobarbital (Nembutal, 40 mg/kg BW, Abbott Laboratories, North Chicago, Ill., USA) injected intraperitoneally. The left jugular vein was cannulated for the infusion of inulin, *p*-aminohippurate, ET and AII. The left carotid artery was cannulated for blood sampling and the measurement of blood pressure. A tracheotomy tube was inserted and mechanical ventilation was started (Rodentventilator model 683, Harverd). The urinary bladder was catheterized through a midline incision in the abdominal wall. Each rat was given an intravenous primary injection (3 ml/kg BW) of inulin (10 g/dl) and *p*-aminohippurate (0.4 g/dl) in saline. Plasma inulin and *p*-aminohippurate concentrations were maintained by a continuous intravenous infusion of 4 g/dl inulin and 0.3 g/dl *p*-aminohippurate in saline at a rate of 0.033 ml/min throughout the experiment. Forty-five minutes were allowed for stabilization: the first 40-min clearance period was then performed. Ten minutes after the injection of ET (0.5 ng/kg BW) or infusion of AII (0.2 μg/kg BW/min), the same 40-min clearance period was performed. After all periods, a blood sample for serum chemistries was obtained.

The mean blood pressure was continuously monitored with a fluid transducer (Nihon-Koden Corp., Tokyo, Japan) connected to a recording instrument (Nihon-Koden). Arterial blood was taken at clearance midpoints. Plasma and urine were frozen and stored until analyzed for inulin and *p*-aminohippurate. The clearance of inulin and *p*-aminohippurate was done by the previously mentioned method [10]. Renal vascular resistance was calculated as follows: renal vascular resistance = mean blood pressure/clearance of *p*-aminohippurate.

The kidneys were fixed in 10% neutral buffered formalin and embedded in paraffin for light-microscopic study. A section (2 μm) was stained with periodic acid-Schiff reagent.To evaluate glomerular cells, a semiquantitative score was used according to the method of Yamamoto et al. [9]. Thirty glomeruli of a 75- to 100-μm diameter from each kidney were examined independently, and the nuclei were counted.

Statistical difference was calculated using an analysis of variance among each of the 3 groups and an unpaired test with the Bonferroni method. A correlation of continuous variables was performed by calculating the correlation coefficient (r).

Results

On day 2 after ATS injection, there was a decrease in the glomerular nuclear counts with mesangiolysis (fig. 1b), compared to the controls (fig. 1a). In contrast, the glomeruli showed a marked hypercellularity on day 8 (fig. 1c). Figure 2 shows the number of nuclei per glomerulus in each stage. The nuclei number on day 2 was significantly lower than that of the controls ($p < 0.005$), and this number on day 8 was significantly higher than that of the controls and day 2 group ($p < 0.005$). The serum concentrations of total protein, blood urea nitrogen, creatinine and sodium were not significantly different among all stages. Mean blood pressure and renal hemodynamics before the injection of AII or ET in all subjects are shown in table 1. Before the injection of ET, there was no difference in the mean blood pressure among all groups. Rats on day 8 showed a reduction of renal function, reflected by the significantly lower levels of renal plasma flow (RPF) of 2.96 ± 0.42 (mean ± SD) ml/min/g kidney and glomerular filtration ratio (GFR) of 0.727 ± 0.079 ml/min/g kidney, and compared with those in the control rats and those of the day 2 group ($p < 0.05$). The renal vascular resistance (RVR) on day 8 was 35.5 ± 6.9 mm Hg min/ml/g kidney, which was significantly higher than that on day 2 ($p < 0.05$), but not significantly different from the control. In addition, the filtration fractions (FF) on day 2 and 8 were significantly lower than that of the control ($p < 0.05$). On day 8, both GFR and RPF were inversely correlated with the number of nuclei in the glomeruli ($r = -0.76$, $p < 0.005$ for GFR; $r = -0.70$, $p < 0.05$ for RPF), while RVR correlated positively with the nuclei number ($R = 0.62$, $p < 0.05$; fig. 3).

Blood pressure began to increase shortly after the injection of ET and remained high after 10–20 min. The degree of blood pressure increase by ET was about 40–45 mm Hg, which was not significantly different from either that of the control, or of the day 2 or day 8 group after ATS injection.

After the injection of ET, reductions in GFR and RPF with elevated RVR were observed in all 3 groups (table 2). The changes of RPF and GFR on day 8 were –33.7 ± 6.2 and –30.0 ± 8.1%, and significantly lower than those of the control and the day 2 group ($p < 0.01$ in GFR; $p < 0.05$ in RPF, fig. 4). There was no significant difference in the changes of GFR, RPF and RVR between the control and day 2 group. However, RVR on day 8 was more greatly changed than that in the control or day 2 group ($p < 0.05$). The decrease in FF did not correlate with the cell number (not shown).

The infusion of AII immediately increased the arterial blood pressure, and its degree of increase was not significantly different among the three 3 groups (table 3). The changes of GFR, RPF and RVR on day 8 were –25.9 ± 4.4, –36.7 ± 9.4 and 133 ± 38%, respectively, which were significantly greater than those in either the day 2 or the control group ($p < 0.05$; fig. 4).

Table 1. Renal hemodynamics in anti-Thy-1 nephritis

	Control	Day 2	Day 8
n	11	12	12
Mean blood pressure, mm Hg	105±11	97±10	102±8
GFR, ml/min/g kidney	0.994±0.080	0.838±0.11[a]	0.727±0.079[a,c]
RPF, ml/min/g kidney	3.50±0.39	3.44±0.53	2.96±0.42[b,c]
RVR, mm Hg ml/min/g kidney	30.0±4.2	28.8±5.6	35.5±6.9[c]
FF	0.287±0.039	0.247±0.036[b]	0.249±0.03[b]

Data are presented as the mean±SD.
[a] $p<0.005$ versus control;
[b] $p<0.05$ versus control;
[c] $p<0.05$ versus day 2.

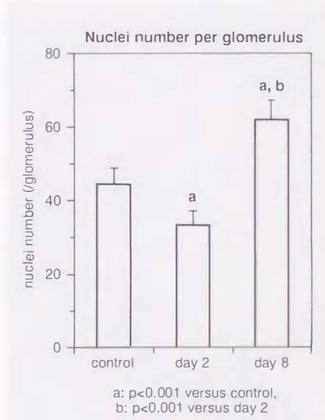

Fig. 2. Nuclei number per glomerulus in each group. The number on day 2 (middle) was significantly lower than that of the control (left, $p < 0.005$). In contrast, the number on day 8 was significantly higher than that of the rest ($p < 0.005$ versus control $p < 0.005$ versus day 2).

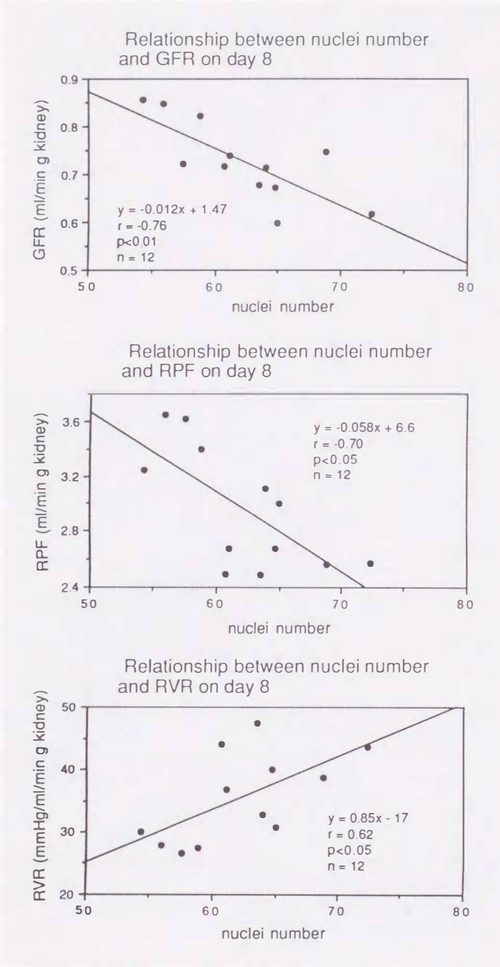

Fig. 3. GFR, RPF and RVR in relation to the number of nuclei in the glomeruli on day 8 after ATS injection.

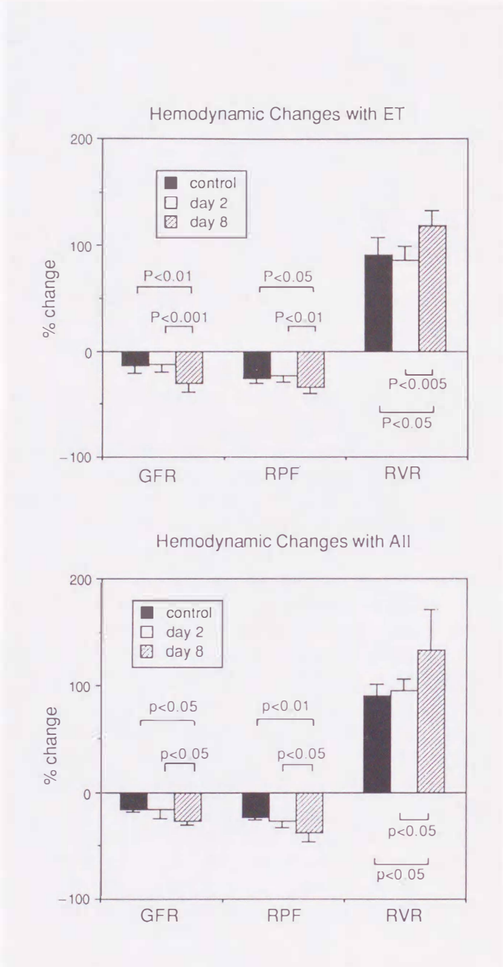

Fig. 4. The changes in GFR, RPF and RVR with injection of ET and AII. Each column represents the mean value of 3 experiments and vertical bars denote SD.

Discussion

This study revealed that the basic values reduction of GFR and RPF and RVR correlated well with the number of glomerular cells in mesangial proliferative nephritis, and also that the decrease in GFR by the injection of either ET or AII was greater in rats with mesangial proliferation than in those with normal kidneys. These results are consistent with the clinical findings that the fall in GFR in response to AII is larger in patients with mesangial proliferation than in healthy subjects [11], suggesting the importance of mesangial proliferation on renal hemodynamics in glomerulonephritis.

Table 2. Hemodynamic change after ET injection

		Control	Day 2	Day 8
n		6	6	6
GFR,	before	0.976±0.10	0.733±0.15[a]	0.675±0.062[a]
ml/min/g kidney	after	0.844±0.070	0.643±0.13[a]	0.470±0.045[a,c]
RPF	before	3.47±0.45	3.06±0.63	2.66±0.18[b]
ml/min/g kidney	after	2.58±0.25	2.30±0.40	1.77±0.25[a,d]
RVR	before	30.0±4.2	28.8±5.6	35.5±6.9
mm Hg ml/min/g kidney	after	60.4±12	63.1±15	86.4±15[b,d]

Data are presented as the mean±SD.
[a] $p<0.005$ versus control; [c] $p<0.005$ versus day 2;
[b] $p<0.05$ versus control; [d] $p<0.05$ versus day 2.

Table 3. Hemodynamic change after AII infusion

		Control	Day 2	Day 8
n		5	6	6
GFR,	before	1.02±0.045	0.899±0.048[b]	0.830±0.061[a,c]
ml/min/g kidney	after	0.854±0.082	0.754±0.071	0.616±0.054[a,c]
RPF	before	3.74±0.72	3.66±0.52	3.42±0.64
ml/min/g kidney	after	2.84±0.42	2.67±0.25	2.11±0.45[b,c]
RVR	before	27.7±6.0	25.6±3.9	30.5±7.0[c]
mm Hg ml/min/g kidney	after	52.6±9.7	50.0±6.9	70.6±18[b,c]

Data are presented as the mean±SD.
[a] $p<0.005$ versus control; [c] $p<0.05$ versus day 2.
[b] $p<0.05$ versus control;

This model was made by intravenous administration of heterologous ATS, which reacts with a Thy-1-like antigen present in rat glomerular mesangial cells [9, 12]. This led to initial lytic lesions and subsequently proliferative/infiltrative lesions in the mesangium. In the ATS-induced glomerular lesion, specific binding of intravenously administered ATS to mesangial cell surface has been found, and the cells which are seen in the proliferative stage are mesangial cells and infiltrating monocytes/macrophages particularly in the areas of mesangial ballooning [9, 12]. Because of this selective change in mesangial cells and the similarity to historical lesions in human glomerulonephritis, we chose this model for glomerulonephritis with mesangial proliferation.

In this study, a good correlation existed between the degree of mesangial proliferation and the decrease in GFR or RPF. The mesangial cells are located between capillaries and possess many of the functional properties of smooth muscle cells [13]. The presence of both actin and myosin is well documented and the contractile properties of cultured mesangial cells are well established [14]. The decreased FF in this nephritis may suggest that the mesangial cells could induce a stronger contractility of glomerular capillary walls, which results in the decrease in GFR associated with the reduction of glomerular surface area. Yamamoto et al. [15] also suggested that mesangial cells might contribute either indirectly or directly to the regulation of afferent arteriolar resistance and plasma flow as well as glomerular capillary hydrostatic pressure in this model. The correlation between RVR and the degree of mesangial proliferation indicates the role of mesangial cells for the reduction of renal hemodynamics in anti-Thy-1 nephritis.

Both AII and ET have been shown to reduce glomerular flow and glomerular ultrafiltration [5, 6, 16, 17]. The former is caused by vasoconstriction of the glomerular arterioles and the latter by apparently acting on contractile elements within the mesangium. The renal hemodynamic response of the kidney with mesangial proliferation to infused ET or AII was modified when compared to those of the control kidney. The reduction of GFR following the injection of both pressor substances was greater in the rats on day 8 than in the control rats. This might be explained by the quantative change of mesangial cells in this nephritis. Blantz et al. [5] have previously reported that the AII-mediated fall in

GFR is due to a direct action on the glomerulus, probably on the mesangial cells, but not on either afferent or efferent arterioles. In the present study, the increase in the number of glomerular cells may thus be essential for the enhanced contractility of glomerular capillaries in anti-Thy-1 nephritis. In addition, the increase in RVR after the injection of these substances was also greater in anti-Thy-1 nephritis. This indicates that the afferent arterioles could more greatly respond to ET or AII in this type of nephritis than to the control. A variety of vasoactive agents such as ET [18], platelet-activating factor [19], renin-like substance [20] and eicosanoids [21] are known to be produced by the mesangial cells or the infiltrating monocytes/macrophages in an inflammatory situation. Either ET or AII might stimulate the mesangial or infiltratory cells in nephritis to secrete vasoactive substances which contributed to the increased afferent arteriolar resistance. Furthermore, the mesangial cells are closely connected to afferent arterioles by gap junctions through Goormaghtigh's cells which are thought to work as a syncytium [22, 23]. These morphological characteristics suggest that mesangial cells play a role in regulating afferent arteriolar resistance. The increased mesangial cells might thus elevate the contractility of afferent arterioles after ET or AII infusion in mesangial proliferative nephritis.

In conclusion, renal hemodynamics in response to the pressor substances were augmented in mesangial proliferative nephritis. The increased number of mesangial cells may, therefore, contribute to the enhanced contractility of the renal vasculatures in anti-Thy-1 nephritis.

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