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Hydrochlorothiazide Increases Efferent Glomerular Arteriolar Resistance in Spontaneously Hypertensive Rats

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Background: Micropuncture studies were performed to determine the intrarenal hemodynamic effects of two conventional antihypertensive agents, hydrochlorothiazide (HCTZ) and hydralazine (HYDR) alone and in combination.

Methods and Results: Male spontaneously hypertensive and normotensive Wistar Kyoto rats (19 weeks old) were treated for 3 weeks with vehicle (control), HCTZ (80 mg/kg/d), HYDR (5 mg/kg/d), or combined therapy (HCTZ 30 mg/kg/d and HYDR 2 mg/kg/d). Each treatment significantly reduced arterial pressure while effective renal plasma flow, glomerular filtration rate and single nephron glomerular filtration rate were unaffected by any treatment in either strain. In spontaneously hypertensive rats HCTZ decreased single nephron plasma flow (111 ± 8 to 84 ± 4 nL/min; $P < .05$) but, despite this reduction, glomerular pressure remained unchanged (51.4 ± 0.7 to 52.1 ± 0.8 mmHg) attributable to increased efferent glomerular resistance (1.58 ± 0.14 to 2.11 ± 0.12 10 U; $P < .05$). By contrast, HYDR increased single nephron plasma flow (to 147 ± 8 nL/min; $P < .01$) and decreased efferent glomerular resistance (to 1.09 ± 0.09 U; $P < .05$). Combined treatment produced responses similar to HCTZ when used alone, thereby nullifying the beneficial efferent glomerular resistance effects: single nephron plasma flow ± fell (to 89 ± 7 nL/min; $P < .05$) and efferent glomerular resistance increased (to 2.05 ± 0.17 U; $P < .05$). In Wistar Kyoto rats, HCTZ and combined treatment had no effect. HCTZ alone induced glomerular ischemia that was associated with efferent glomerular arteriolar constriction in these spontaneously hypertensive rats.

Conclusions: These findings provide a possible explanation for the lack of improved renal target-organ damage in controlled multicenter trials employing thiazide diuretics.

Key words: intrarenal hemodynamics, renal micropuncture, spontaneously hypertensive rats, Wistar Kyoto rats, diuretics.

Long-term antihypertensive therapy in large controlled multicenter trials, employing diuretics alone or with hydralazine (HYDR), has dramatically reduced the prevalence of stroke, congestive heart failure, and coronary heart disease, but it apparently has not affected renal complications.[1] Moreover, it is unclear at this time, whether any one class of antihypertensive agents is preferable for patients with hypertension and renal functional involvement. Several reports have suggested that either glomerular hypertension with consequent hyperfiltration or glomerular ischemia may promote glomerular injury and nephrosclerosis in experimentally uninephrectomized rats.[2,4] Only a few micropuncture studies were concerned with the more prolonged effects of antihypertensive agents in the spontaneously hypertensive rats with intact kidneys and none using diuretics alone. Recent study from our laboratory suggested that prolonged treatment with an angiotensin-converting enzyme inhibitor increased single nephron plasma flow and reduced glomerular capillary pressure associated with reduced afferent and efferent arteriolar resistances.[6] Glomerular capillary pressure is regulated by renal perfusion pressure as well as by the delicate balance between afferent and efferent glomerular arteriolar resistances and may be

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affected by vasoactive agents. In this study, we investigated the prolonged effects of two traditional antihypertensive agents, hydrochlorothiazide (HCTZ) and HYDR, used alone or in combination, on the intrarenal circulation of the spontaneously hypertensive rats and its normotensive control, the Wistar-Kyoto rat.

Materials and Methods

Male spontaneously hypertensive rats and Wistar-Kyoto rats (Charles River Laboratories) were each divided into four equal subgroups. One subgroup was treated with the vehicle (serving as a control). The others were treated with HCTZ (80 mg/kg/d), HYDR (5 mg/kg/d), or the combination of HCTZ (30 mg/kg/d) and HYDR (2 mg/kg/d). Each dose (or dose combination) was selected to reduce arterial pressure to the same extent, and the dose of HCTZ was precisely the same as that used by other workers for similar studies.[7,8] All rats were allowed free access to water and standard rat chow. Treatment was initiated at 19 weeks of age by gastric gavage for 3 weeks. HCTZ was administrated once a day; rats treated with HYDR alone or with combination therapy were administered the drugs in two equally divided doses 12 hours apart. Preliminary experiments had established that these dosing schedules were necessary to maintain a prolonged reduction in arterial pressure that was equivalent in all treatment subgroups, as measured in the awake rat by tail-cuff plethysmography. The selection of a 3-week treatment period provided was chosen to be comparable with other reports from our laboratory describing 3-week treatment on cardiac mass and function and systemic and regional hemodynamics in the spontaneously hypertensive rats and Wistar-Kyoto rats.[9] The experimental protocol had been approved by our institutional animal care committee. Each rat was studied with renal micropuncture techniques following treatment as we reported earlier from our laboratory.[6,10–12] After overnight fasting which permitted free access to water prior to study, the rats were anesthetized with Inactin (70 mg/kg; intraperitoneally; Byk Gulden, Konstanz, Germany), after which they were placed on a heating pad to maintain body temperature at 37°C throughout the study. The right femoral artery was cannulated, and approximately 120 μL of arterial blood was collected to serve as a baseline blank value for p-aminohippurate assay. This arterial catheter was also used for subsequent blood sampling and to measure directly mean arterial pressure and heart rate. Catheters were also inserted into the left jugular and right femoral veins. Tracheostomy was performed to ensure adequate and stable ventilation. The left jugular vein was used to infuse [^{3}H]-inulin (850 μCi/mL) (Du Pont-New England Nuclear, Boston, MA) at a rate 0.1 mL/100g body weight/h. The right femoral vein was used for infusion of saline containing 5.6% P-aminehippurate assay (Merck Sharp and Dohme, West Point, PA) at a rate of 0.2 mL/100g body weight/h and saline containing 12.5% albumin at a rate of 0.8 mL/100g body weight/h for the initial 60 minutes of the preparatory surgical procedures; the maintenance rate was 0.15 mL/100 g body weight/h thereafter.

The left kidney was exposed through a subcostal incision. Once isolated, it was separated from surrounding fat, and the left ureter was catheterized with PE-10 tubing. The kidney was then mounted on a lucite holder, on which 2% warm agar was dripped to form a saline or mineral oil well. The bladder was cannulated to measure the right kidney urine volume. Urine was collected over two 30-minute periods, and blood samples were withdrawn at the midpoint of each collection. If the glomerular filtration rate (GFR) of the two kidneys differed by more than 30% the results from those experiments were discarded. The following micropuncture measurements were obtained: (1) efferent glomerular blood was withdrawn by direct puncture of two to three superficially located "star-vessels"; (2) precisely timed (90 seconds) samples of fluid were collected from four to six randomly selected superficial proximal tubules for determination of single nephron GFR (SNGFR); and (3) efferent arteriolar (P_E), proximal tubular (P_T), and stop-flow pressures were measured by a servo-nulling system (Instrumentation for Physiology and Medicine, Inc., San Diego, CA).[13] The proximal tubular and efferent arteriolar measurements were obtained from proximal convoluted tubules and "star-vessels," respectively, both selected randomly. Because spontaneously hypertensive rats glomerular capillaries are not located on the renal surface, glomerular capillary pressure was considered to be the sum of the stop-flow pressure and systemic colloid osmotic pressure.[14] These efferent arteriolar, proximal tubular, and stop-flow pressure measurements were made at least three times and average values were calculated.

After the rats were killed with excess pentobarbital, the hearts were removed immediately, and the atria were carefully dissected free from the ventricles. The free wall remained as part of the left ventricle. Wet masses of the blotted ventricles were carefully determined on a grammatic balance. The left and right ventricular masses were expressed as the ratio of ventricular weight to body weight (mg/g).

The tubular fluid, urine, and plasma samples were counted in a beta-scintillation counter for [^{3}H]inulin activity by placing these samples in 10 mL scintillation vials (Bio-Safe II, Mount Prospect, IL). GFR was calculated using the standard clearance formula. Hematocrit was determined from all arterial samples. Effective renal plasma flow (ERPF) was determined from P-aminohippurate clearance and effective blood flow (ERBF) was calculated as follows: Effective renal blood flow = Effective renal plasma flow/(1 – Hematocrit A), where Hematocrit A was arterial Hematocrit. All data for ERPF, ERBF, and GFR were normalized using the left kidney weight after decapsulation at the conclusion of the study. Filtration fraction was determined as follows: Filtration fraction = GFR/ERPF. Total renal vascular resistance was calculated from the equation renal vascular resistance = mean arterial pressure/Effective renal blood flow, where mean arterial pressure was considered identical to the renal arterial pressure. Arterial plasma protein concentration (C_A) was measured refractometrically, and plasma osmotic pressure (Π_A) was calculated from the Landis-Pappenheimer equation.[13] Glomerular capillary pressure (P_G) under stopped-flow conditions, was calculated from the equation:

$$P_G = \text{stop-flow pressure} + \Pi_A.$$

The pressure gradient across glomerular capillary wall, ΔP, was calculated from

$$\Delta P = P_G - P_T.$$

Single nephron filtration fraction was calculated from

$$\text{Single nephron fraction flow} = 1 - [\text{Hematocrit A}\,(1 - \text{Hematocrit E})/\text{Hematocrit E}(1 - \text{Hematocrit A})],$$

where Hematocrit E is the efferent arteriole hematocrit.[14,15] SNGFR was calculated from tubular fluid radioactivity divided by plasma radioactivity. Single nephron plasma flow SNPF was calculated as Single nephron plasma flow = SNGFR/single nephron fraction flow. Single nephron blood flow was single nephron blood flow = single nephron plasma flow/(1 – Hematocrit A). Protein concentration of efferent arteriole (C_E) was calculated from $C_E = C_A/(1 - \text{single nephron filtration fraction})$, and efferent arteriolar osmotic pressure (Π_E) was also calculated from the Landis-Pappenheimer equation. Afferent and efferent glomerular arteriolar resistances (R_A, R_E) were calculated as follows: $R_A = (\text{mean arterial pressure} - P_G)/\text{single nephron blood flow} \times 7.962 \times 10^{10}$, and $R_E = (P_G - P_E)/(\text{single nephron blood flow} - \text{SNGFR}) \times 7.962 \times 10^{10}$. The ultrafiltration coefficient (Kf) was calculated as follows: $Kf = SNGFR/(\Delta P - \Delta\Pi) / 60$, where $\Delta\Pi$ is the transmembrane colloid osmotic pressure difference and was calculated according to the equation detailed by Deen et al. and modified by Arendshorst and Gottschalk.[17,18]

To compare the effects of treatment on the spontaneously hypertensive rats or Wistar-Kyoto rats, a one-way analysis of variance, followed by Duncan test, was performed between the control and three treated groups. For comparison of the baseline control levels of Wistar-Kyoto rats and spontaneously hypertensive rats, an unpaired *t*-test was employed. All data were expressed as the mean ± 1 SEM; and a probability level of 5% was considered to be of statistical significance.

Results

There was no significant difference in body weight between Wistar-Kyoto rats and spontaneously hypertensive rats whether treated or untreated. The control left and total ventricular weights of spontaneously hypertensive rats were greater than those of Wistar-Kyoto rats, but there was no difference in right ventricular weight between the two strains. Left ventricular weight was unaffected by the 3 weeks of treatment with HCTZ or HYDR in spontaneously hypertensive rats; however, the combined treatment significantly reduced left ventricular weight. Wistar-Kyoto rats ventricular weights were unaffected by any treatment (Table 1).

Control mean arterial pressure and renal vascular resistance were significantly greater in spontaneously hypertensive rats than Wistar-Kyoto rats. Moreover, the baseline values for effective renal plasma flow, effective renal blood flow, GFR, and filtration fraction of the two strains were not different; however renal vascular resistance of spontaneously hypertensive rats was significantly increased (Table 2; Fig. 1). With respect to glomerular dynamics, SNPF, SNGFR, single nephron filtration fraction, and all intrarenal pressure measurements were not different between the two strains; however, afferent glomerular arteriolar resistances of the spontaneously hypertensive rats was increased ($P < .01$; Table 3; Fig. 2).

All three treatment protocols significantly reduced mean arterial pressure in both strains, but the absolute reductions were greater in spontaneously hypertensive rats than in the Wistar-Kyoto rats. The HCTZ treatment increased UV in spontaneously hypertensive rats, but this was not significantly so in Wistar-Kyoto rats. Heart rate remained unchanged with each treat-

Table 1. Body Weight and Heart Weight

	WKY				SHR			
	Control	HCTZ	HYDR	HCTZ + HYDR	Control	HCTZ	HYDR	HCTZ + HYDR
Number	10	8	9	7	10	8	8	8
Body Weight (g)	361 ± 9	340 ± 11	379 ± 5	334 ± 6	366 ± 6	339 ± 13	356 ± 4	337 ± 8
LV Weight (mg/g)	2.31 ± 0.12	2.15 ± 0.04	2.19 ± 0.04	2.13 ± 0.10	2.70 ± 0.06†	2.60 ± 0.05	2.57 ± 0.04	2.45 ± 0.5*
RV Weight (mg/g)	0.57 ± 0.03	0.61 ± 0.03	0.67 ± 0.04	0.66 ± 0.07	0.57 ± 0.02	0.62 ± 0.04	0.63 ± 0.04	0.66 ± 0.04
Total Ventricular Weight (mg/g)	2.73 ± 0.03	2.77 ± 0.06	2.85 ± 0.07	2.79 ± 0.11	3.28 ± 0.06†	3.22 ± 0.06	3.20 ± 0.06	3.11 ± 0.08

*$P < .01$ compared with respective control. †$P < .01$ compared with WKY control.
Values are mean ± 1 SEM. HYDR, hydralazine; HCTZ, hydrochlorothiazide; LV, left ventricular; RV, right ventricular.

ment. In Wistar-Kyoto rats, neither HCTZ nor HYDR altered whole kidney hemodynamics, although the combination treatment significantly decreased filtration fraction. In spontaneously hypertensive rats, however, none of the three treatments affected ERPF, ERBF, and GFR; although renal vascular resistance significantly decreased with HYDR and with the combination treatment (Table 2; Fig. 1). In Wistar-Kyoto rats, all glomerular dynamic values (except for stop-flow pressure and glomerular capillary pressure of HYDR) remained unchanged with treatments, however, stop-flow pressure and glomerular capillary pressure were significantly decreased with HYDR (Table 3). Afferent arteriolar blood hematocrit was greater in spontaneously hypertensive rats than in the Wistar-Kyoto rats. In spontaneously hypertensive rats, SNGFR, single nephron filtration fraction, and all intrarenal pressure measurements except for SNPF remained unaffected with HCTZ; SNPF decreased significantly by HCTZ and combined therapy, and increased to the same level as with WKY by HYDR (Table 3; Fig. 2). Thus, the unchanged glomerular capillary pressure was associated with a significant increase in efferent glomerular resistance and an increased SNPF with HYDR was associated with significant decrease in SNFF and afferent glomerular resistance. Despite the increase of SNPF ($P < .01$), stop-flow pressure ($P < .01$) and glomerular capillary pressure ($P < .05$) were significantly decreased in the HYDR group. It could be a result of a reduced efferent glomerular resistance ($P < .05$). The effects of combined treatment showed similar tendency to those of HCTZ alone; hence, SNPF decreased and efferent glomerular resistance increased significantly ($P < .05$). Afferent arteriolar blood protein concentration was unchanged compared with respective HYDR (Table 3).

Discussion

Antihypertensive therapy over the past several decades has reduced the prevalence of stroke, coronary heart disease, and congestive heart failure in the hypertensive population, whereas renal complications have continued to increase.[1] Moreover, diuretics have been the first choice of antihypertensive agents for over 35 years,[9,20] and their effects on the intrarenal circulation remain unclear. In this study we investigated the prolonged effects (3 weeks) of HCTZ, alone or with

Table 2. Systemic and Renal Hemodynamics

	WKY				SHR			
	Control	HCTZ	HYDR	HCTZ + HYDR	Control	HCTZ	HYDR	HCTZ + HYDR
MAP (mmHg)	124 ± 3	109 ± 3*	104 ± 4*	107 ± 6*	190 ± 4†	164 ± 3*	154 ± 9*	147 ± 5*
HR (beats/min)	363 ± 7	340 ± 6	367 ± 9	356 ± 8	352 ± 6	330 ± 15	350 ± 10	348 ± 4
ERPF (mL/min/g)	3.58 ± 0.26	3.61 ± 0.26	4.40 ± 0.33	4.31 ± 0.35	3.18 ± 0.12	2.61 ± 0.22	3.62 ± 0.29	3.51 ± 0.40
ERBF (mL/min/g)	7.01 ± 0.48	6.89 ± 0.49	8.42 ± 0.64	7.93 ± 0.66	6.66 ± 0.25	5.58 ± 0.52	7.34 ± 0.53	6.93 ± 0.75
GFR (mL/min/g)	1.03 ± 0.09	0.88 ± 0.07	1.00 ± 0.09	0.86 ± 0.07	0.96 ± 0.06	0.79 ± 0.07	0.95 ± 0.10	0.80 ± 0.09
FF (%)	29.0 ± 2.2	25.5 ± 2.9	22.9 ± 1.6	20.1 ± 1.4†	30.6 ± 2.1	31.0 ± 2.8	26.3 ± 2.3	23.1 ± 2.0
RVR (U)	18.9 ± 2.0	16.4 ± 1.5	12.9 ± 1.2	14.5 ± 2.2	28.8 ± 1.1‡	31.0 ± 2.6	21.6 ± 1.7†	22.6 ± 2.8†
UV (µL/min/g)	4.5 ± 0.5	6.2 ± 0.5	5.7 ± 1.0	6.2 ± 0.6	4.2 ± 0.3	7.3 ± 1.5†	6.0 ± 0.6	6.8 ± 0.6†

*$P < .01$, †$P < .05$ compared with respective control. ‡$P < .01$ compared with WKY control.
Values are mean ± 1 SEM. HYDR, hydralazine; HCTZ, hydrochlorothiazide; MAP, mean arterial pressure; HR, heart rate; ERPF, effective renal plasma flow; ERBF, effective renal blood flow; GFR, glomerular filtration rate; FF, filtration fraction; RVR, renal vascular resistance.

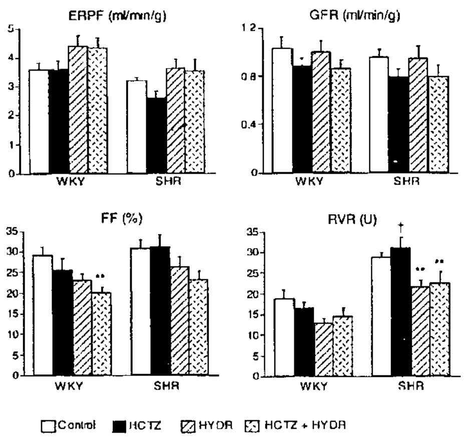

Fig. 1. Renal hemodynamics in WKY and SHR. Values are mean ± 1 SEM. HCTZ, hydrochlorothiazide, HYDR, hydraladine **P < .05 compared with respective control, †P < .01 compared with WKY control.

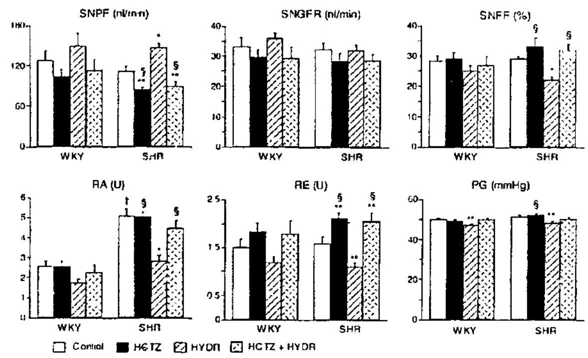

Fig. 2. Glomerular dynamics in WKY and SHR. Values are mean ± 1 SEM. HCTZ, hydrochlorothiazide; HYDR, hydraladine. *P < .01, **P < .05 compared with respective control. §P < .01 compared wtih respective control. †P < .01 compared with respective control.

HYDR, on intrarenal circulation using classical renal micropuncture techniques.

Studies involving several animal models with renal disease have shown that glomerular hyperfiltration and hypertension have been related to protein leakage, progressive functional deterioration, and glomerular sclerosis.[2,3,17] These rat experimental models, however, required removal of one kidney and remnant tissue destruction in the other. The mechanism and demonstration of glomerular sclerosis in the naturally occurring model of hypertension of the spontaneously hypertensive rats having both kidneys intact, is also unclear, but one possible mechanism that has been suggested is glomerular ischemia with exacerbation of an accelerated renin angiotensin system.[5] One recent study from our laboratory suggested that 3-week treatment with an ACE inhibitor might reduce the hemodynamic risk in spontaneously hypertensive rats with intact kidneys[6]; however, a multicenter trial showed that serum creatinine levels had increased in the diuretic-treated patients.[21]

The results of the present study demonstrated that 3 weeks of therapy with HCTZ reduced SNPF and increased efferent glomerular resistance in the spontaneously hypertensive rats even though the pretreatment SNPF was normal. A similar treatment protocol

Table 3. Glomerular Dynamics

	WKY				SHR			
	Control	HCTZ	HYDR	HCTZ + HYDR	Control	HCTZ	HYDR	HCTZ + HYDR
SNGFR (nL/min)	33.0 ± 3.1	29.3 ± 2.9	35.8 ± 2.0	29.2 ± 3.7	32.1 ± 2.4	28.0 ± 2.9	31.7 ± 1.9	28.4 ± 2.3
SNPF (nL/min)	127 ± 15	103 ± 11	150 ± 19	113 ± 16	111 ± 8	84 ± 4**§	147 ± 8*	89 ± 7**§
SNFF (%)	28 ± 2	29 ± 2	25 ± 2	27 ± 3	29 ± 1	33 ± 3§	22 ± 1*	32 ± 2§
P_T (mmHg)	12.3 ± 0.2	11.3 ± 0.4	12.0 ± 0.4	12.4 ± 0.3	11.8 ± 0.5	12.5 ± 0.5	12.8 ± 0.5	11.9 ± 0.2
P_E (mmHg)	14.7 ± 0.3	13.5 ± 0.4	13.6 ± 0.4	14.5 ± 0.4	13.7 ± 0.6	12.6 ± 0.6	13.0 ± 0.7	13.6 ± 0.6
Π_A (mmHg)	19.3 ± 0.4	19.9 ± 0.3	20.2 ± 0.5	20.4 ± 0.5	20.5 ± 0.4	20.9 ± 0.6	20.4 ± 0.4	20.8 ± 0.3
Π_E (mmHg)	31.8 ± 1.0	34.3 ± 1.9	32.1 ± 1.1	33.8 ± 2.5	34.3 ± 0.4	41.6 ± 4.5§§	30.1 ± 1.1	39.4 ± 2.3§§
SFP (mmHg)	30.6 ± 0.7	29.1 ± 0.9§§	26.9 ± 0.2*	29.4 ± 0.5§§	30.9 ± 0.6	31.2 ± 0.8§	27.9 ± 0.6*	29.5 ± 0.7
P_G (mmHg)	49.9 ± 0.8	49.0 ± 0.9	47.1 ± 0.5**	49.9 ± 0.7	51.4 ± 0.7	52.1 ± 0.8§	48.3 ± 0.7**	50.3 ± 0.7
ΔP (mmHg)	37.5 ± 0.7	37.8 ± 1.2	35.1 ± 0.5	37.5 ± 0.7	39.2 ± 0.8	39.6 ± 0.9§	35.5 ± 0.8*	38.5 ± 0.6§§
R_A (U)	2.56 ± 0.27	2.58 ± 0.28	1.71 ± 0.20	2.25 ± 0.39	5.05 ± 0.35†	5.02 ± 0.23§	2.83 ± 0.27*	4.47 ± 0.38§
R_E (U)	1.49 ± 0.20	1.82 ± 0.19	1.19 ± 0.12	1.78 ± 0.28	1.58 ± 0.14	2.11 ± 0.12**§	1.09 ± 0.09**	2.05 ± 0.17**§
Kf (nL/s/mmHg)	0.047 ± 0.006	0.053 ± 0.011	0.068 ± 0.005	0.047 ± 0.009	0.044 ± 0.004	0.047 ± 0.009	0.052 ± 0.004	0.051 ± 0.007
HctA (%)	48.9 ± 0.8	47.7 ± 0.6	47.8 ± 0.7	45.9 ± 0.2	52.3 ± 0.7†	53.0 ± 0.6§§	50.8 ± 0.7	50.4 ± 0.6‡
Ca (g/dL)	5.8 ± 0.1	5.9 ± 0.1	6.0 ± 0.1	6.0 ± 0.1	6.0 ± 0.1	6.1 ± 0.1	6.0 ± 0.1	6.1 ± 0.1
Ce (g/dL)	8.1 ± 0.2	8.4 ± 0.3	8.1 ± 0.2	8.3 ± 0.4	8.4 ± 0.1	9.3 ± 0.6§	7.7 ± 0.2	9.1 ± 0.3§

*P < .01, **P < .05 compared with respective control. §P < .01, §§P < .05 compared with respective HYDR. ‡P < .05 compared with respective HCTZ. †P < .01 compared with WKY control.

Values are mean ± 1 SEM. HYDR, hydralazine; HCTZ, hydrochlorothiazide.

with HYDR increased SNPF and reduced efferent glomerular resistance; however, co-treatment with HCTZ nullified these potentially beneficial intrarenal hemodynamic effects. A prior hemodynamic study from our laboratory, using radionuclide-tagged microspheres, demonstrated a reduced total renal vascular resistance in conscious and unrestrained spontaneously hypertensive rats also treated with HYDR for 3 weeks.[9] However, in spite of this increased SNPF produced by HYDR, glomerular pressure decreased significantly as a result of the significant reduction of efferent glomerular resistance in the spontaneously hypertensive rats. These findings suggest that HYDR might reduce the risk of renal injury and, hence, be of therapeutic benefit. On the other hand, Kohara et al.[22] showed that it failed to improve proteinuria, renal function, and renal histological findings in spontaneously hypertensive rats previously subjected to five-sixths nephrectomy.

Left ventricular hypertrophy is an independent risk factor in patients with essential hypertension in part, the result of cardiac arrhythmias, left ventricle ischemia, or failure.[23] With respect to ventricular hypertrophy in the present study, HYDR did not alter cardiac mass in either spontaneously hypertensive rats or Wistar-Kyoto rats despite the significant pressure and afterload reduction. These data substantiate earlier work that indicated that this vasodilator did not reverse cardiac hypertrophy.[9,24] In previous studies, using experimental rat models of cardiac failure, the intrarenal circulation was altered to a similar degree as in experimental models of hypertension, as manifested by efferent glomerular resistance and glomerular pressure elevations.[10,11] Clearly, further studies would be necessary to investigate the long-term effects of HYDR on intrarenal circulation in hypertension with intact kidneys.

In contrast to the foregoing findings with HYDR, HCTZ was studied using a higher dose when used alone and at a lower dose in combination with HYDR. Both treatments significantly reduced SNPF in spontaneously hypertensive rats. In spite of this reduced SNPF with the thiazide, the glomerular pressure remained unchanged, most likely reflecting the significant increase in efferent glomerular resistance. The mechanism(s) for this increased efferent glomerular resistance resulting from HCTZ cannot be explained by the present data, although the local or systemic generation of angiotensin II by diuretic-induced stimulation of the renin-angiotensin system is a real possibility.[25,26] Generalized renal vasoconstriction (such as produced by angiotensin II), increases glomerular pressure[27]; and this elevated glomerular pressure declines with angiotensin-converting enzyme inhibition therapy that reduces that proteinuria.[28] It follows then, that a prolonged increase in efferent glomerular resistance could promote proteinuria, glomerular pressure, and renal damage. In the present study, we did not demonstrate any changes in whole kidney function; however, HCTZ diminished SNPF and increased glomerular arteriolar resistance. This points to the value of micropuncture study, since these glomerular hemodynamic changes are similar to those postulated for developing hypertensive nephrosclerosis in patients with essential hypertension when the extent of hemodynamic involvement is less severe. Therefore, glomerular ischemia with diminished SNPF and efferent arteriolar constriction might be factors in development of glomerular sclerosis with more prolonged HCTZ treatment. Alternatively, Benstein et al.[29] reported that HCTZ promoted proteinuria in uninephrectomized spontaneously hypertensive rats suggesting that the provoked hypokalemia and hyperlipidemia might contribute to the renal injury.

The consequences of the combined low-dose treatment with HCTZ and HYDR were similar to those of HCTZ used alone: decreased SNPF and increased efferent glomerular resistance. In our preliminary studies it was not possible to reduce arterial pressure in the spontaneously hypertensive rats with the low-dose HCTZ when used alone, but by adding the HYDR, with the lower dose, arterial pressure was reduced to the same degree as with the higher dose of HYDR when used alone. In the presence of HCTZ, however, the HYDR failed to offset the increased efferent arteriole resistance in the SHR.

HCTZ did not affect ERPF, but it did reduce SNPF significantly. This could be explained by a mechanism of uneven distribution of renal plasma flow produced by the thiazide. With volume depletion, renal plasma flow and filtration rate have been shown to be reduced in superficial cortical glomeruli but maintained in juxtamedullary glomeruli.[25,31] In the present study, the combination treatment produced the same effects as when the thiazide was used alone.

Several studies have been reported concerning the effects of "triple antihypertensive therapy" on the renal circulation.[3,7,28] With triple therapy, reserpine is added to our combination of HCTZ and HYDR, but the antihypertensive effect of reserpine in the spontaneously hypertensive rats was weak.[32] Nevertheless, it is possible to compare the results of our combination treatment with those of the triple therapy. The triple therapy study reported by Dworkin et al.[3] reduced glomerular pressure and injury in uninephrectomized spontaneously hypertensive rats. This reduction in glomerular pressure resulted from a decreased efferent glomerular resistance a response that is consistent with a direct vascular effect of the drug on the efferent arteriole. These same investigators reported the effects of HCTZ in uninephrectomized spontaneously hypertensive rats[29] using a dose that was similar to that in their previous study. In that study HCTZ did not decrease arterial pressure and SNPF, glomerular pressure, and afferent and efferent glomerular resistance were not changed. In the present report, HCTZ reduced mean arteriolar pressure and increased. The differences between the earlier report[3] and ours could be related to differences in the doses employed, which were greater in our study. Perhaps that need for a higher dose in our study reflects the presence of the two intact kidneys and the uninephrectomy with a remnant kidney in the latter study.[15] Further differences might also be due to the experimental models. Uninephrectomy increase glomerular pressure and plasma renal flow and, therefore, exacerbated renal injury. In contrast to the uninephrectomized rat, our control spontaneously hypertensive rats demonstrated normal kidney function.

These results add further credence to the thesis that HCTZ increases efferent resistance and that this factor could thereby contribute to ultimate renal injury in the patient with essential hypertension treated for many years with this agent. We investigated the renal pathologic changes in the spontaneously hypertensive rats kidneys in this pilot study; however, histopathology was normal in each group. Pathologic changes in spontaneously hypertensive rats have demonstrated rapid progression of disease from 45 to 65 weeks,[33] suggesting that pathological changes had not yet occurred in the 22-week spontaneously hypertensive rats employed in this study. Nevertheless, Freis et al.[34] reported that they had been able to prevent glomerulosclerosis in the spontaneously hypertensive rats treated with the triple therapy during the first year of life, examining them pathologically at 52 weeks.[34] By way of contrast, however, Feld et al.[7] reported that, despite continuous control of arterial pressure with triple therapy in the spontaneously hypertensive rats, glomerulosclerosis developed by 100 weeks of age. Thus, proteinuria progressively worsened and renal function deteriorated significantly by 1 year of age, and the investigators, therefore, concluded that triple therapy could only delay, not prevent, renal injury in spontaneously hypertensive rats with two kidneys. Failure of arterial pressure reduction to completely prevent the renal functional and morphological changes in the spontaneously hypertensive rats suggests participation of other factors; however, since these workers were unable to determine glomerular hemodynamics, the relationship between glomerular pressure and renal injury in older spontaneously hypertensive rats with two kidneys continues to remain unresolved.

In summary, the present data demonstrate reduced SNPF and increased efferent glomerular arteriolar resistance after 3 weeks of thiazide treatment in the spontaneously hypertensive rats. This arteriolar constriction was not prevented with co-treatment with the direct-acting vasodilator hydralazine. Moreover, these intrarenal vascular changes are similar to those early changes postulated to promote glomerular sclerosis in patients with essential hypertension. The data therefore, suggest that prolonged use of hydrochlorothiazide could participate in the renal injury of hypertensive rats and might also contribute to the progressive deterioration of renal function in at least some patients with essential hypertension who may be subjected to prolonged diuretic therapy.

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