Original Article

Developmental Activity of the Renin-Angiotensin System during the "Critical Period" Modulates Later L-NAME–Induced Hypertension and Renal Injury

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The incidence of hypertension and hypertensive renal disease is increasing worldwide, and new strategies to prevent these diseases need to be investigated. The aims of this study were 1) to examine if transient exposure to an angiotensin receptor blocker (ARB) during an early period in hypertension development confers protection against subsequent worsening of hypertension and renal injury induced by the NO synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME), and 2) conversely, to examine the effects of transient exposure to angiotensin II (Ang II) during the same period. First, spontaneously hypertensive rats (SHR) were treated transiently from age 3 to 10 weeks with an ARB (candesartan cilexetil), a calcium channel antagonist or a vasodilator, then taken off treatment for 2 months. Administration of L-NAME at age 18 weeks caused severe hypertension and renal injury. However, the rats that had been exposed to the ARB not only had a lower blood pressure, but also failed to show signs of renal injury or increase of oxidative stress. Furthermore, the elevation of components of the renin-angiotensin-aldosterone system was also suppressed in these rats. In the second study, Wistar-Kyoto rats (WKY) and SHR were exposed to Ang II from age 4 to 8 weeks. The follow-up showed that the blood pressures in the WKY remained elevated compared to controls, while the SHR had heightened increases in blood pressure, renal renin mRNA, and urinary 8-hyroxydeoxyguanosine after L-NAME administration. Together, these experiments demonstrate that transient treatment of rats during an early phase in the development of hypertension with an ARB suppresses the renin-angiotensin-aldosterone system and confers long-term protection against subsequent L-NAME–induced renal injury and increases in renal oxidative stress. Conversely, developmental exposure to Ang II during this "critical" period had the opposite effect, predisposing rats to higher blood pressure, renal injury, and oxidative stress after L-NAME administration. (*Hypertens Res* 2007; 30: 63–75)

***Key Words*: angiotensin, hypertension, renal injury, renin-angiotensin-aldosterone system, oxidative stress**

Introduction

The incidence of hypertension was estimated as approximately 26.4% in 2004 and continues to increase worldwide, with a projected value of 29.2% by 2025 (*1*). Renal injury is an important complication of hypertension, and hypertensive nephrosclerosis is the leading cause of end-stage renal disease in many countries. It is therefore an important research goal to develop new methods to prevent the incidence of hypertension and hypertensive renal injury.

It has been shown that the renin-angiotensin system plays a

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Received May 1, 2006; Accepted in revised form September 4, 2006.

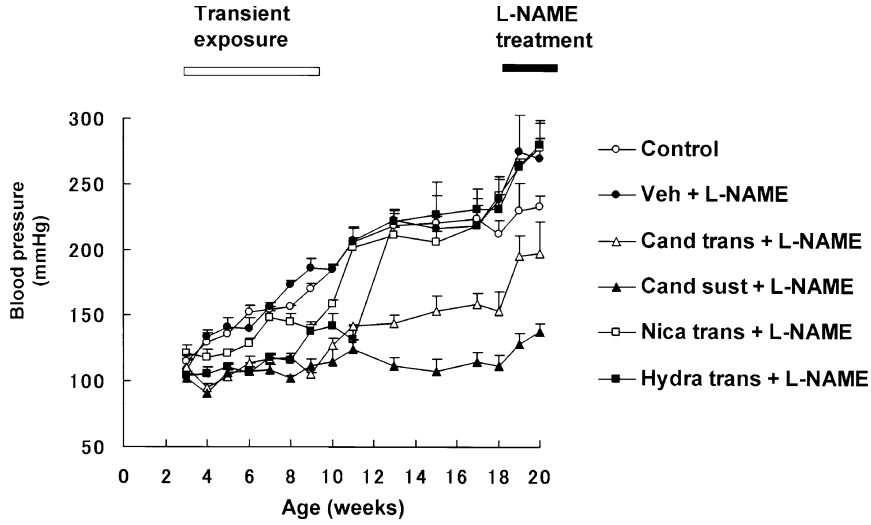

Fig. 1. *Effects of transient exposure to the ARB candesartan cilexetil on blood pressure in L-NAME–treated SHR. Control: untreated; Veh: rats treated with vehicle (tap water); Cand trans: rats treated with candesartan cilexetil from age 3 to 10 weeks; Cand sust: rats treated with candesartan cilexetil from age 3 to age 21 weeks; Nica trans: rats treated with nicardipine from age 3 to age 10 weeks; Hydra trans: rats treated with hydralazine from age 3 to age 10 weeks; L-NAME: treated with L-NAME from age 18 to age 21 weeks. Blood pressures in the candesartan-treated groups were significantly reduced compared to the other four groups at all time points after week 12 (*$p < 0.01$*, symbols omitted for clarity).*

Table 1. Systolic Blood Pressure (SBP) and Heart Rate (HR) in the Different Groups in Experiment 1

	Group number					
	1 Veh	2 Veh	3 Cand trans	4 Cand sust	5 Nica trans	6 Hydra trans
L-NAME treatment	–	+	+	+	+	+
SBP (mmHg) 3 w	115±5	109±7	111±6	102±3	121±6	104±4
HR (beats/min) 3 w	483±23	489±35	494±18	515±17	488±16	512±18
SBP (mmHg) 10 w	185±4	185±3	127±6**	115±9**	159±3*	142±9**
HR (beats/min) 10 w	387±15	392±24	367±21	410±11	401±21	424±30
SBP (mmHg) 18 w	212±11	238±18	154±14**	112±8**	241±13	231±11
HR (beats/min) 18 w	336±18	326±13	378±22	418±18	364±27	374±19
SBP (mmHg) 20 w	233±8*	270±15	197±25**	138±6**	278±19	280±19
HR (beats/min) 20 w	339±18	381±24	309±18	340±17	323±21	348±34

Veh: rats treated with vehicle (tap water); Cand trans: rats treated with candesartan cilexetil from age 3 to 10 weeks; Cand sust: rats treated with candesartan cilexetil from age 3 to age 21 weeks; Nica trans: rats treated with nicardipine from age 3 to age 10 weeks; Hydra trans: rats treated with hydralazine from age 3 to age 10 weeks. *$p<0.05$, **$p<0.01$ *vs*. L-NAME (+) Veh. w, weeks.

central role in the pathogenesis of both hypertension and hypertensive renal injury. Recent studies have also highlighted a role for oxidative stress in the pathophysiology of hypertension and hypertensive end-organ damage (*2*, *3*). Therefore, the development of interventions to prevent excessive activation of the renin-angiotensin system, and to permanently attenuate the development of oxidative stress, would be important steps toward the ultimate goal of preventing hypertension and hypertensive renal disease.

Previously, we reported that treatment of stroke-prone spontaneously hypertensive rats (SHRSP) or Dahl salt-sensitive (Dahl-S) rats during a critical period in the development of hypertension (from age 3 to 10 weeks) with an angiotensin inhibitor resulted in an attenuation of hypertension and renal damage (*4*, *5*). Both of these rat models can be categorized as genetic (*i.e.*, hereditary) models of hypertensive renal disease.

The aim of the present study was to examine whether transient exposure to an angiotensin receptor blocker (ARB) can confer long-term protection against the later development of acquired hypertension and renal damage induced by the administration of a nitric oxide (NO) synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME), and to examine the

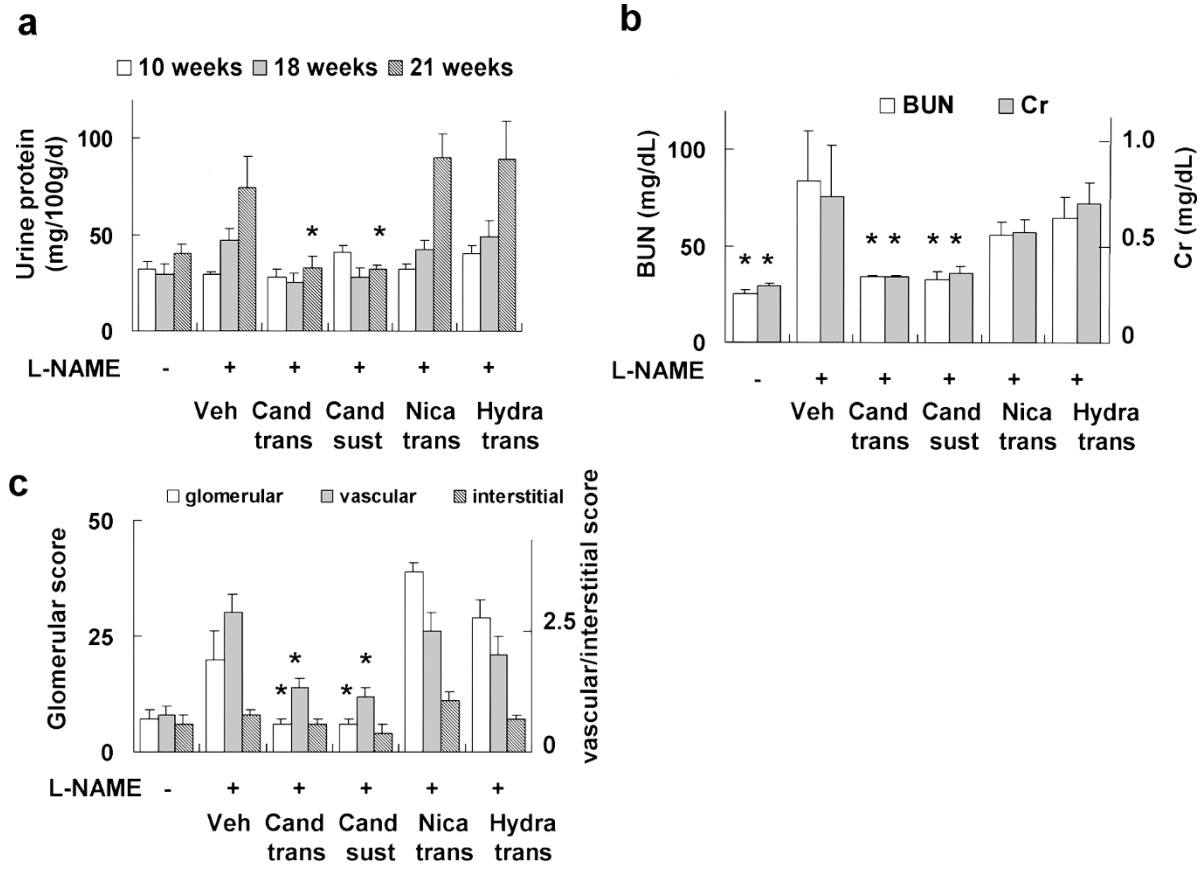

Fig. 2. *Effects of transient exposure to the ARB candesartan cilexetil on indices of renal injury after L-NAME administration. (a) Urine protein; (b) blood urea nitrogen (BUN) and creatinine (Cr); and (c) renal histology score. Abbreviations of the different groups are as in Fig. 1.* *p < *0.05* vs. *Veh + L-NAME.*

changes in the renin-angiotensin-aldosterone system and oxidative stress in the treated rats. The second objective of this study was to perform the "mirror image" experiment—namely, to examine the effects of transient administration of Ang II to Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHR) on the subsequent course of hypertension and L-NAME–induced renal injury in these animals.

Methods

Animal Treatment Protocols

Experiment 1

The studies were conducted using 3-week-old male SHR (SHR/Izm maintained by Sankyo Laboratory Services, Tokyo, Japan). All experiments were performed in accordance with the Animal Experimentation Guidelines of the Keio University School of Medicine.

The rats were randomly divided into 6 treatment groups as follows: Rats in group 1 ($n=5$) were untreated SHR. Rats in group 2 ($n=9$) were treated with vehicle (tap water) from age 3 to 10 weeks. From age 18 to 21 weeks, L-NAME was administered in the drinking water at a dose of 50 mg/l. Rats in group 3 ($n=9$) and group 4 ($n=6$) were treated with the ARB candesartan cilexetil dissolved in the drinking water at a dose of 1 mg/kg/day. In the case of group 3, candesartan cilexetil was administered transiently from age 3 to 10 weeks, and then the rats were taken off medication (transient exposure). In contrast, rats in group 4 were treated with candesartan cilexetil continuously from age 3 to 21 weeks (sustained exposure). In both groups, L-NAME was administered from age 18 to 21 weeks. Rats in groups 5 ($n=6$) and 6 ($n=7$) were treated transiently with the calcium channel blocker nicardipine (50 mg/kg/day) or the vasodilator hydralazine (25 mg/kg/day) in the drinking water from age 3 to 10 weeks, and then the rats were taken off medication. Subsequently, L-NAME was administered from age 18 to 21 weeks.

Experiment 2

Studies were conducted using 3-week-old male WKY (WKY/Izm) (group 1 [$n=6$] and group 2 [$n=10$]) and SHR (SHR/Izm) (group 3 [$n=7$] and group 4 [$n=19$]). At the age of 4 weeks, osmotic minipumps were inserted subcutaneously

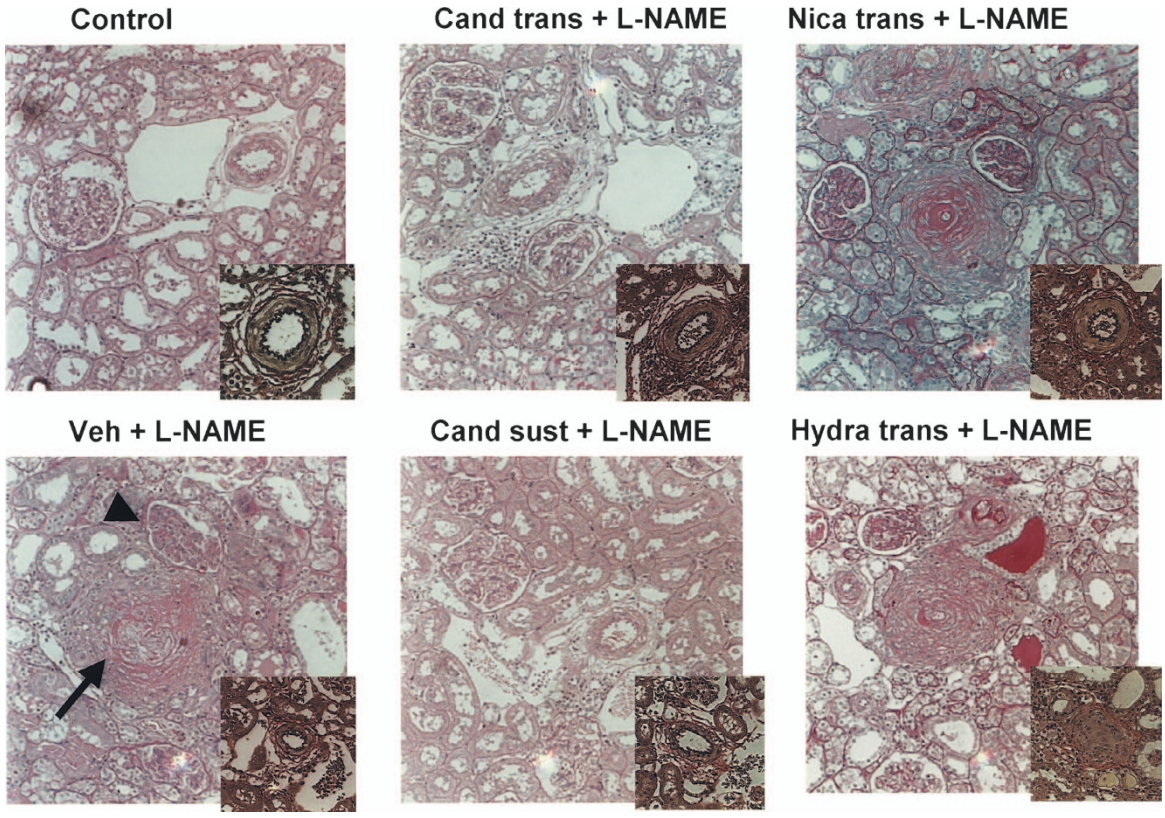

Fig. 3. *Light microscopy of renal histological sections in the different groups. Representative photomicrographs of paraformaldehyde-fixed sections stained with PAS are shown. Original magnification: × 200. The arrowhead indicates an area of glomerulosclerosis. The arrow indicates hyperplasia of small- to medium-sized arteries. Insets show details of vascular injury (Elastica van Gieson stain, original magnification: × 400). Abbreviations of the different groups are as in Fig. 1.*

Table 2. Parameters of Cardiovascular Hypertrophy in the Different Groups in Experiment 1

	Group number					
	1 Veh	2 Veh	3 Cand trans	4 Cand sust	5 Nica trans	6 Hydra trans
L-NAME treatment	–	+	+	+	+	+
Body weight (BW, g)	319±21	350±12	322±14	255±21	340±14	322±18
Heart weight (HW, g)	1.47±0.06	1.49±0.14	1.43±0.04	1.02±0.03**	1.61±0.04	1.39±0.06
HW/BW × 100	0.43±0.04	0.47±0.03	0.43±0.03	0.41±0.03	0.48±0.02	0.41±0.02
Media thickness (μm)	0.14±0.01	0.17±0.01	0.15±0.02	0.11±0.01†	0.17±0.01	0.18±0.01
Media/lumen × 100	8.1±0.4	9.9±0.4	8.7±0.7	7.3±0.5	9.0±0.5	9.8±0.4

Veh: rats treated with vehicle (tap water); Cand trans: rats treated with candesartan cilexetil from age 3 to 10 weeks; Cand sust: rats treated with candesartan cilexetil from age 3 to age 21 weeks; Nica trans: rats treated with nicardipine from age 3 to age 10 weeks; Hydra trans: rats treated with hydralazine from age 3 to age 10 weeks. **$p<0.01$ *vs*. L-NAME (+) Veh; †$p<0.05$ *vs*. L-NAME (+) Hydra trans.

under ether anesthesia. The minipumps were set to infuse vehicle (saline) (groups 1 and 3) or Ang II (60 ng/min) (groups 2 and 4) from age 4 to 8 weeks. Following the infusions, the pumps were removed and weighed to ensure delivery of the contents. The rats were observed without treatments until they were 18 weeks of age, and then L-NAME (5 mg/kg/day) was administered in the drinking water from age 18 to 20 weeks.

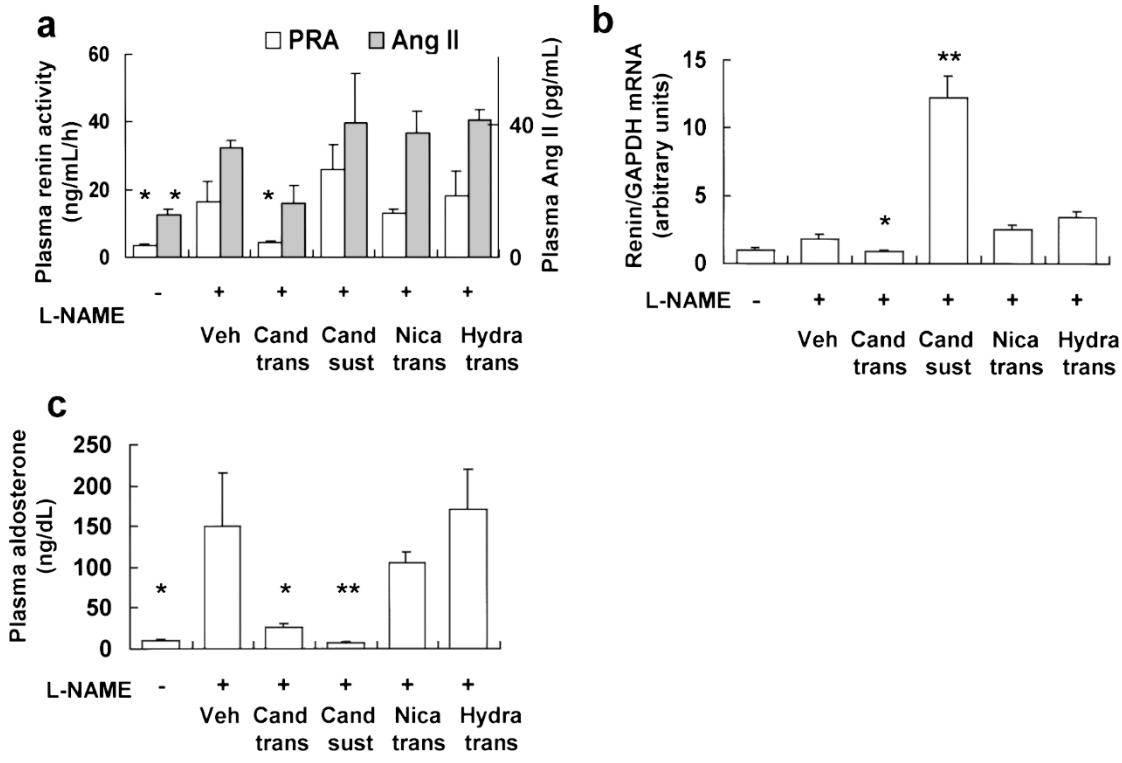

Fig. 4. *Effects of transient exposure to the ARB candesartan cilexetil on the renin-angiotensin-aldosterone system after L-NAME administration. (a) Plasma renin activity (PRA) and plasma angiotensin II (Ang II); (b) renal renin mRNA; (c) plasma aldosterone. Abbreviations of the different groups are as in Fig. 1.* *p < *0.05,* **p < *0.01* vs. *Veh + L-NAME.*

Assays

The systolic blood pressure (SBP) and heart rate (HR) of awake animals were measured by tail-cuff plethysmography using a Natsume KN-210 manometer (Natsume Inc., Tokyo, Japan). Twenty-four hour urine collection was performed in metabolic cages, and urinary protein concentrations were determined using the Lowry method. Blood urea nitrogen (BUN), serum creatinine, total cholesterol, and triglycerides were measured using an autoanalyzer. Plasma renin activity (PRA) was determined by radioimmunoassay of angiotensin I formed by incubation of plasma for 1 h at 37°C, while plasma angiotensin II (Ang II) and plasma aldosterone concentrations (PAC) were determined by radioimmunoassay (*4*, *6*).

Histological Studies

The kidneys and thoracic aortae were fixed in 4% paraformaldehyde, then embedded in paraffin blocks. Histologic sections from the rat kidneys were stained with PAS and Elastica van Gieson, while the sections from the aortae were stained with Azan. Slides were examined by light microscopy, and the renal histopathological changes were scored as described previously (*4*).

Real-Time Reverse Transcription–Polymerase Chain Reaction Analysis of Renal Gene Expressions

Total RNA was purified from the kidneys by the acid guanidine–phenol–chloroform method, and quantified by measurement of absorbance at 260 nm in a spectrophotometer. Renin, Ang II converting enzyme (ACE), and angiotensinogen mRNA were analyzed by real-time reverse transcription–polymerase chain reaction (RT-PCR) using previously reported primers and probes, and normalized to the corresponding values of GAPDH mRNA (*7*).

Assay of Markers of Oxidative Stress

Plasma lipid peroxides were measured using the hemoglobin methylene blue method (*8*). Plasma thiobarbituric acid reactive substances (TBARS) were measured using a TBARS assay kit (Zeptometrix Corporation, Buffalo, USA) in accordance with the manufacturer's instructions. Urinary 8-hydroxydeoxyguanosine (8-OHdG) concentrations were determined by an enzyme-linked immunosorbent assay (Japan Institute for the Control of Aging, Shizuoka, Japan).

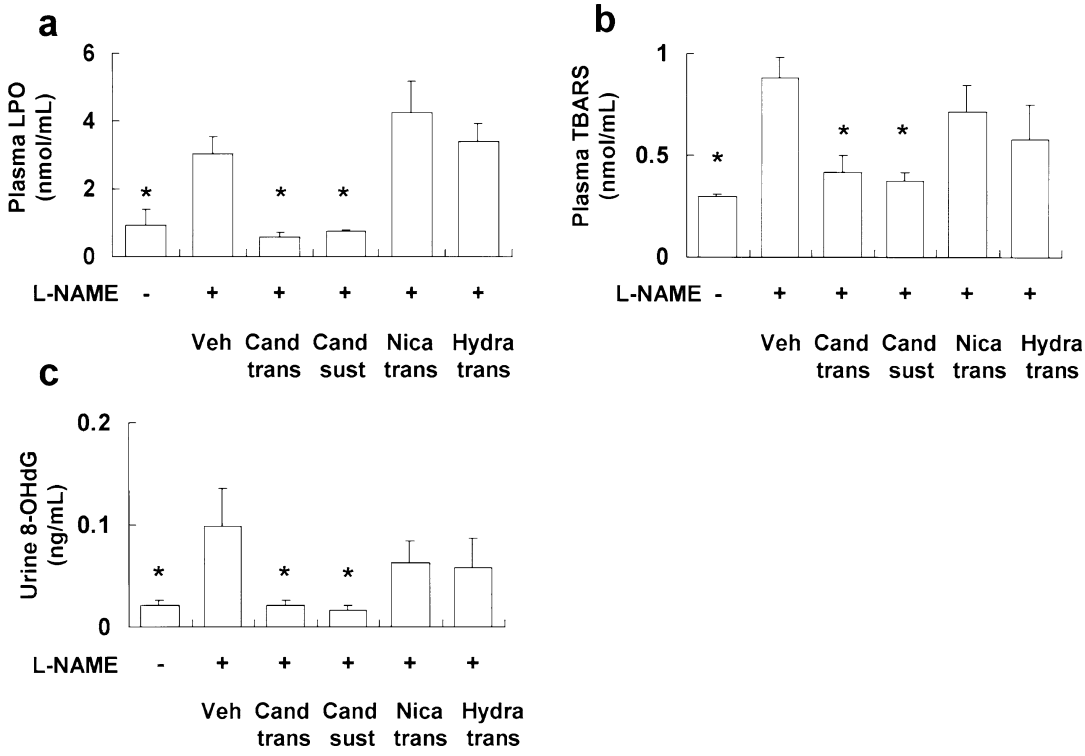

Fig. 5. *Effects of transient exposure to the ARB candesartan cilexetil on markers of oxidative stress after L-NAME treatment. (a) Plasma lipid peroxide (LPO) concentrations; (b) plasma TBARS concentrations; (c) urinary 8-hydroxydeoxyguanosine (8-OHdG) concentrations. Abbreviations of the different groups are as in Fig. 1.* *p < *0.05* vs. *Veh + L-NAME.*

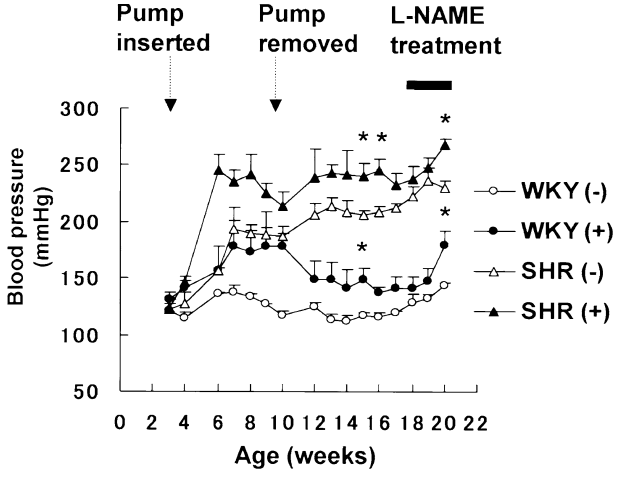

Fig. 6. *Effects of transient exposure to Ang II or vehicle on blood pressure in WKY and SHR. WKY (–), (+): WKY infused with vehicle (saline) or Ang II, respectively, from age 4 to age 8 weeks. SHR (–), (+): SHR infused with vehicle (saline) or Ang II, respectively, from age 4 to age 8 weeks.* *p < *0.05* vs. *the corresponding (–) group. Blood pressures in the Ang II–infused groups were significantly increased compared to those in the vehicle-infused groups during the infusion period (*p < *0.01, symbols omitted for clarity).*

Materials

Candesartan cilexetil and nicardipine hydrochloride were provided by Takeda Chemical Industries Ltd. (Osaka, Japan) and Yamanouchi Pharmaceuticals Co. Ltd. (Tokyo, Japan), respectively. Real-time RT-PCR reagents were obtained from Applied Biosystems (Foster City, USA). Other chemicals were from Sigma (St. Louis, USA), unless specified otherwise.

Statistics

Results were expressed as the mean±SEM. Statistical comparisons were made by ANOVA followed by Scheffe's post-hoc test. p values <0.05 were considered to be statistically significant.

Results

Effects of Transient Exposure to ARB on Blood Pressure in L-NAME–Treated SHR

The changes in the SBP in the different groups are shown in Fig. 1. In the control group, the SBP was approximately 110 mmHg at age 3 weeks, but rose thereafter to reach a value of

Table 3. Systolic Blood Pressure (SBP) and Heart Rate (HR) in the Different Groups in Experiment 2

	Group			
	1 WKY	2 WKY	3 SHR	4 SHR
Ang II	–	+	–	+
SBP (mmHg) 3 w	121±6	131±6	122±9	124±9
HR (beats/min) 3 w	448±23	430±11	443±16	472±10
SBP (mmHg) 10 w	117±4	178±18**	187±2**	214±13**,†
HR (beats/min) 10 w	438±10	431±27	410±32	384±24
SBP (mmHg) 18 w	129±7	142±9	222±9**	238±11**
HR (beats/min) 18 w	352±18	381±23	356±14	388±16
SBP (mmHg) 20 w	144±3	180±12*	230±6**	268±5**,†
HR (beats/min) 20 w	398±13	397±28	360±7	351±15

*$p<0.05$, **$p<0.01$ *vs*. WKY Ang II (–); †$p<0.05$ *vs*. SHR Ang II (–). WKY, Wistar Kyoto rats; SHR, spontaneously hypertensive rats; w, weeks.

185±3 mmHg by age 10 weeks. In the rats treated with candesartan, hydralazine, or nicardipine from age 3 to 10 weeks, the SBP was reduced during the treatment period compared to the values in the control group. Following discontinuation of the drug, the SBP in the hydralazine- and nicardipine-treated groups rose to values similar to those in the control group (200 mmHg or greater). In contrast, the SBP in the candesartan (transient)-treated groups remained significantly reduced (154±18 mmHg at age 18 weeks). In the rats with sustained treatment with candesartan, the SBP was maintained at less than 150 mmHg. There were no significant differences in the HR among the different groups (Table 1).

Following administration of L-NAME, the SBP in the control group rose to 270±8 mmHg. Similar values were seen in the nicardipine (transient) and hydralazine (transient) groups (278±19 mmHg and 290±19 mmHg, respectively). In contrast, the SBP in the candesartan (transient) group was suppressed to a value of 197±15 mmHg.

Effects of Transient Exposure to ARB on Proteinuria, Renal, and Cardiovascular Changes

The development of proteinuria, renal dysfunction, and renal injury is depicted in Fig. 2. The urine protein levels were not significantly different at age 18 weeks compared to age 10 weeks in any of the groups. Following the administration of L-NAME in the drinking water (starting at age 18 weeks), the urine protein excretion was increased in the vehicle group. Similarly, the urine protein excretion in the nicardipine (transient) and hydralazine (transient) groups was increased approximately 2-fold after L-NAME treatment. In contrast, the urine protein in the rats treated transiently with candesartan did not become elevated after L-NAME treatment.

These results were confirmed by measurement of the BUN and serum creatinine. Both of these parameters were increased in the L-NAME–treated vehicle group, whereas in the rats treated transiently with candesartan, the values were similar to those in the rats not treated with L-NAME.

Examination of renal histology revealed a similar contrast between the rats treated transiently with candesartan, and the rats treated transiently with nicardipine or hydralazine. As shown in Figs. 2c and 3, the histological findings in the normal (control) SHR at age 21 weeks did not reveal any remarkable renal injury. Treatment with L-NAME caused histological changes consisting of glomerulosclerosis and vascular injury. These changes were suppressed in the candesartan (transient) group, as well as the candesartan (sustained) group.

Concerning cardiac and aortic hypertrophy, both transient and sustained treatment with candesartan caused a trend towards decreased heart weight/body weight and media/lumen ratios, the difference in heart weight and aortic media thickness reaching statistical significance in the candesartan (sustained) group (Table 2).

Effects of Transient Exposure to ARB on the Renin-Angiotensin-Aldosterone System

Administration of L-NAME was found to cause significant increases in the PRA, plasma Ang II, and PAC as compared with the values in the control rats, and a similar trend was seen for renin mRNA, as shown in Fig. 4. These increases were suppressed in the rats treated transiently with candesartan, consistent with a sustained suppression of the renin-angiotensin-aldosterone system. In the rats belonging to the candesartan (sustained) treatment group, both the PRA and Ang II levels remained elevated, while the level of PAC was suppressed, consistent with peripheral blockade of the Ang II type 1 (AT1) receptors. In contrast, no major differences in ACE or angiotensinogen mRNA levels were found (data not shown).

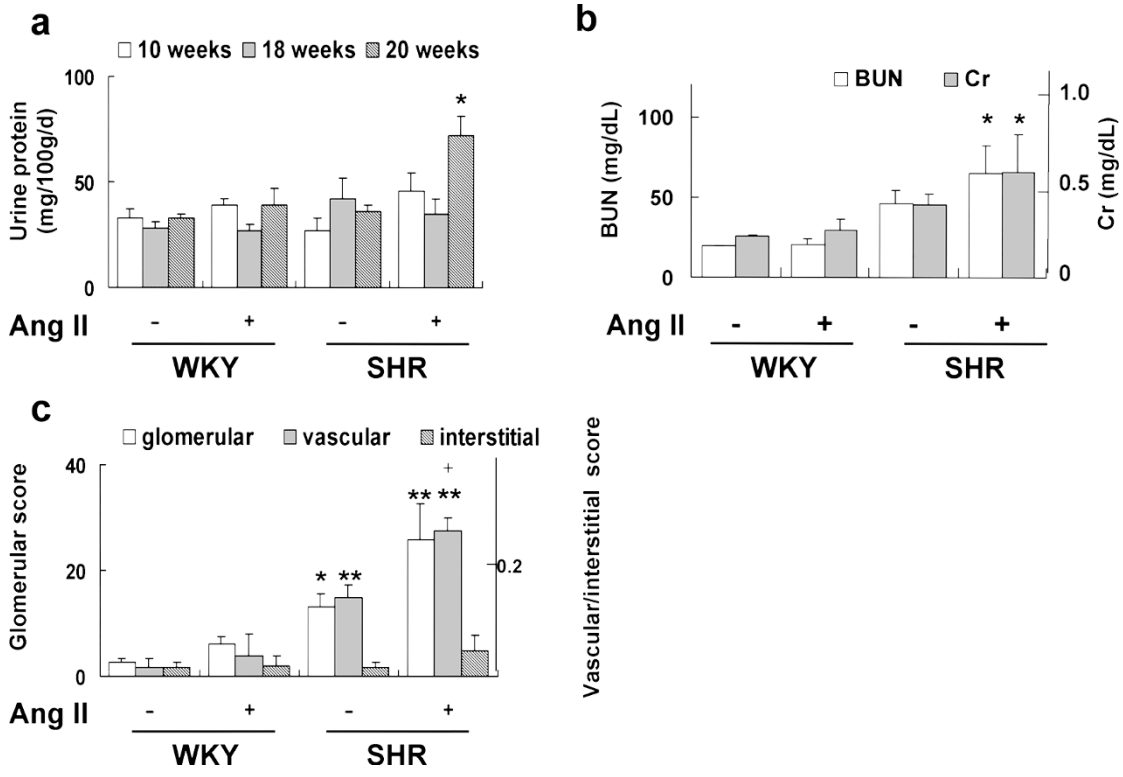

Fig. 7. *Effects of transient exposure to Ang II or vehicle on indices of renal injury after L-NAME administration. (a) Urine protein; (b) blood urea nitrogen (BUN) and creatinine (Cr); (c) renal histology score. Abbreviations of the different groups are as in Fig. 6.* *p < *0.05,* **p < *0.01* vs. *WKY (–).* $^{+}$p < *0.05* vs. *SHR (–).*

Effects of Transient Exposure to ARB on Markers of Oxidative Stress

Markers of oxidative stress were assessed in the plasma and urine of the different groups. Treatment with L-NAME was associated with a 3-fold increase in the plasma levels of lipid peroxides and TBARS, and a 5-fold increase in the urinary 8-OHdG concentrations. These changes were suppressed in the rats treated transiently with candesartan (Fig. 5).

Effects of Transient Exposure to Ang II on Blood Pressure in WKY and SHR

The effects of exposure of the animals to Ang II during the period from age 4 to 8 weeks on the subsequent blood pressure and L-NAME–induced renal changes were examined. As shown in Fig. 6, insertion of osmotic minipumps delivering Ang II to the WKY and SHR caused an increase in SBP in the order of 40–80 mmHg. Following the removal of the minipumps, the SBP in the WKY decreased, but not to the same levels as seen in the WKY exposed to vehicle (saline) alone. The values of SBP after 2 weeks' administration of L-NAME were 144±3 mmHg in the WKY rats exposed to saline vehicle (Ang II (–) rats), and 180±12 mmHg in the rats receiving Ang II (Ang II (+) rats [$p < 0.05$]). Similarly, in the SHR, exposure to Ang II from age 4 to 8 weeks was associated with significant increases in the SBP both before and after L-NAME treatment (230±6 mmHg and 268±5 mmHg after L-NAME treatment in the Ang II (–) and Ang II (+) groups, respectively; $p < 0.05$) (Table 3).

Effects of Transient Exposure to Ang II on Mortality Rates and Renal Injury in WKY and SHR

Administration of L-NAME to the Ang II–exposed SHR resulted in a strikingly high mortality rate (14 out of 19 rats = 74%). Because of the high mortality rate, L-NAME was administered for only 2 weeks in Experiment 2 (as compared to 3 weeks in Experiment 1).

L-NAME treatment for 2 weeks was not associated with statistically significant increases in proteinuria, BUN/creatinine, or renal histological changes in the WKY (Figs. 7, 8). In the case of the SHR, the rats which had been exposed to Ang II showed the highest urine protein excretion level and BUN/creatinine among the four groups. Although the proteinuria and increases in BUN/creatinine tended to be greater in the SHR Ang II (+) rats compared to the Ang II (–) group, the differences did not reach statistical significance. However, histological examination confirmed a significantly greater glomerular and vascular injury score in the SHR as compared

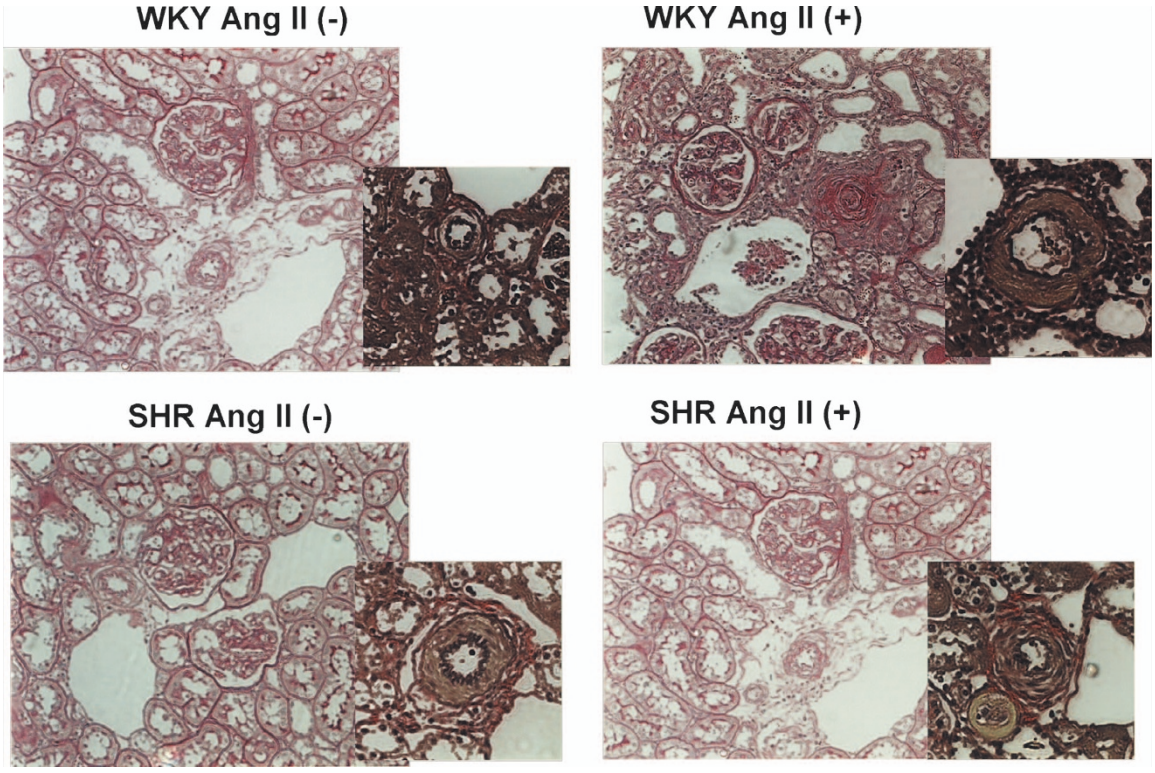

Fig. 8. *Light microscopy of renal histological sections in the different groups. Representative photomicrographs of paraformaldehyde-fixed sections stained with PAS are shown. Original magnification: × 200. Insets show details of the vascular injury (Elastica van Gieson stain, original magnification: × 400). Abbreviations of the different groups are as in Fig. 6.*

Table 4. Parameters of Cardiovascular Hypertrophy in the Different Groups in Experiment 2

	Group			
	1 WKY	2 WKY	3 SHR	4 SHR
Ang II	–	+	–	+
Body weight (BW, g)	381±3	381±8	292±10**	290±12**
Heart weight (HW, g)	1.46±0.04	1.48±0.06	1.68±0.10	1.62±0.10
HW/BW × 100	0.38±0.01	0.39±0.02	0.58±0.03**	0.56±0.03**
Kidney weight (KW, g)	1.42±0.03	1.34±0.03	1.18±0.03**	1.33±0.04††
KW/BW × 100	0.37±0.01	0.35±0.01	0.40±0.01	0.46±0.02**,†
Media thickness (μm)	0.13±0.01	0.14±.0.1	0.15±0.02	0.19±0.02**
Media/lumen × 100	9.2±0.3	9.7±0.5	9.6±0.2	10.2±2.0

**$p<0.01$ *vs*. WKY Ang II (–); †$p<0.05$, ††$p<0.01$ *vs*. SHR Ang II (–). WKY, Wistar Kyoto rats; SHR, spontaneously hypertensive rats; w, weeks.

to that in the WKY, and also a significantly higher vascular injury score in the SHR Ang II (+) rats as compared to that in the control SHR Ang II (–) rats; a similar trend was noted in the WKY (Figs. 7c, 8). Moreover, the kidney weights and kidney weight/body weight ratios were both significantly increased in the SHR Ang II (+) rats as compared to the SHR Ang II (–) rats (Table 4).

Effects of Transient Exposure to Ang II on the Renin-Angiotensin-Aldosterone System and Urine Markers of Oxidative Stress in WKY and SHR

The levels of PRA, Ang II, and PAC were found to be significantly higher in the SHR compared to the WKY (Fig. 9). Moreover, the levels of PRA, renin mRNA, plasma Ang II,

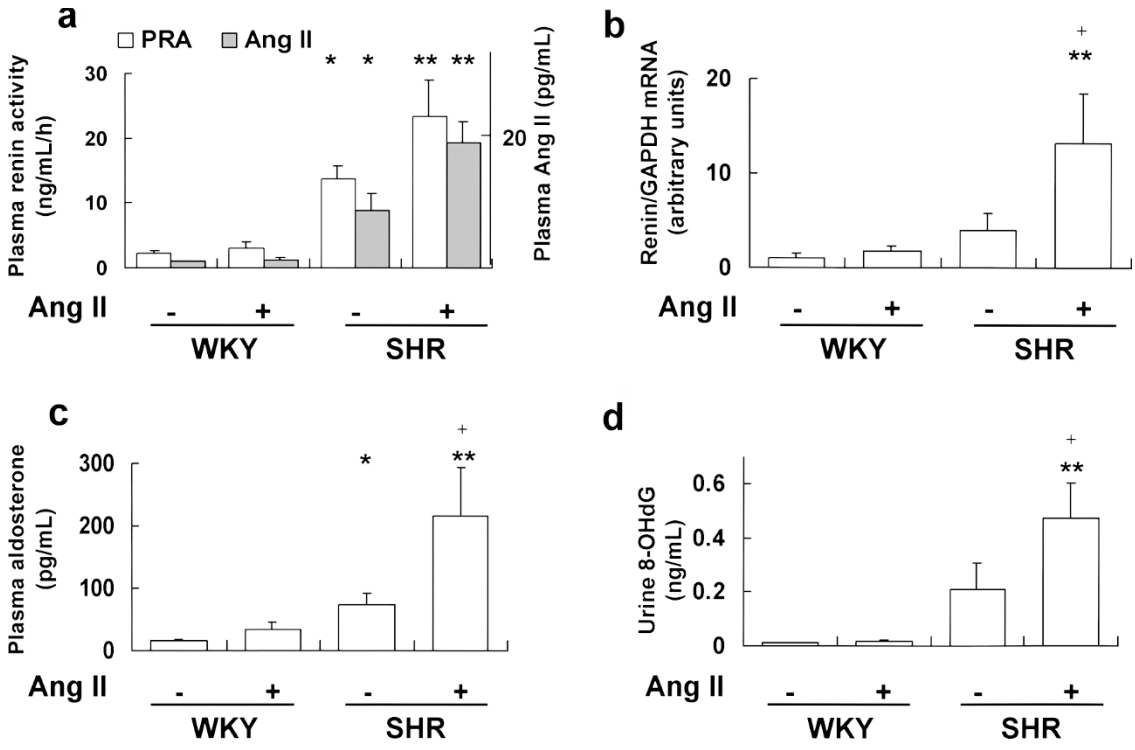

Fig. 9. *Effects of transient exposure to Ang II or vehicle on the renin-angiotensin-aldosterone system and urinary oxidative stress after L-NAME administration. (a) Plasma renin activity (PRA) and plasma angiotensin II (Ang II); (b) renal renin mRNA; (c) plasma aldosterone; and (d) urine 8-hydroxydeoxyguanosine (8-OHdG) concentrations.* *$p < 0.05$, **$p < 0.01$ vs. *WKY (–).* $^{+}p < 0.05$ vs. *SHR (–). Abbreviations of the different groups are as in Fig. 6.*

and PAC all tended to be higher in the SHR Ang II (+) rats compared to the Ang II (–) rats, with the difference reaching statistical significance for the renin mRNA and plasma aldosterone levels. Urinary 8-OHdG levels were also significantly higher in the SHR Ang II (+) rats compared to the Ang II (–) rats.

Discussion

Treatment of rats with the NO synthase (NOS) inhibitor L-NAME causes generalized endothelial dysfunction and inhibition of NO-induced vasodilation, resulting in an acquired form of hypertension (*9*). L-NAME–treated rats also develop renal histological changes characteristic of hypertensive injury, such as glomerulosclerosis and vascular injury, and thus the L-NAME model is a useful tool to study both the development and the treatment of renal lesions resembling those found in human hypertension (*10*).

Previous studies from our and other laboratories have shown that transient exposure of genetically hypertensive rats to a renin-angiotensin system inhibitor during a "critical period" in hypertension development results in the suppression of subsequent hypertension. Following the initial studies on the development of hypertension by Harrap, Berecek, and Morton's groups and others (*11–13*), we reported that transient treatment with an ARB can also attenuate the development of hereditary (genetically induced) hypertensive renal injury in both the SHRSP and Dahl-S rat models. This treatment has also been reported to attenuate the progression of renal injury in diabetic OLETF rats (*14*). In terms of sexual development, this age in rats (age 3 to 10 weeks) corresponds to early adolescence (3 to 16 years) in humans. However, when considered in terms of hypertension development, this period may be considered as being equivalent to the phase of "prehypertension" in humans (*4*, *5*, *15*). Age 18 weeks in rats corresponds to a period of established hypertension in humans, *i.e.*, middle age or later.

The first aim of the present study was to examine if exposure to an ARB during this "critical period" could confer protection against the development of the acquired form of hypertension and renal injury induced by the NOS inhibitor L-NAME in later life. SHR were treated with the ARB candesartan cilexetil, the calcium channel blocker nicardipine, or the vasodilator hydralazine from age 3 to 10 weeks. The rats were then taken off treatment and observed for a further 2 months. We found that the blood pressure in rats transiently treated with ARB remained reduced during the 2 months off treatment. From age 18 weeks, the rats were treated with L-

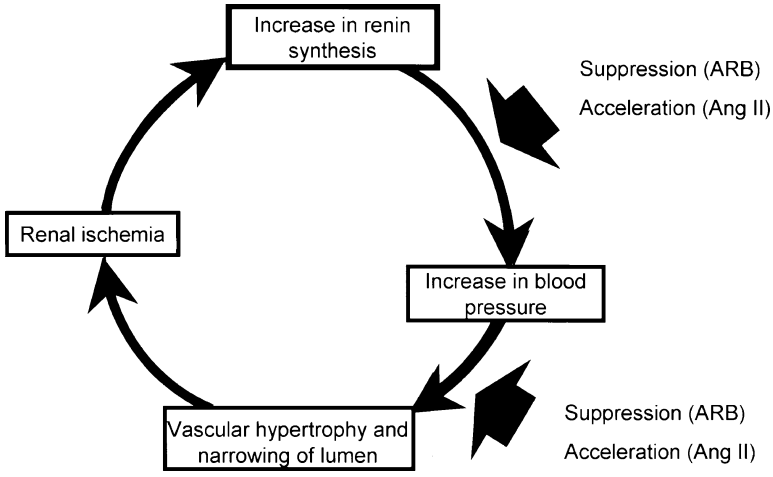

Fig. 10. *The "reno-vascular amplifier" hypothesis.*

NAME with the aim of inducing hypertension and renal injury, and the extent of the injury was assessed by measurement of proteinuria, BUN and creatinine, and histological examination. We found that hypertensive renal injury was clearly evident in the rats exposed to nicardipine or hydralazine, but was almost completely suppressed in the rats transiently exposed to candesartan.

These findings suggested that early exposure to ARB could confer long-standing protection against subsequently acquired hypertension and renal injury in later life. To clarify the importance of Ang II during this developmental period, we further examined the effects of administration of Ang II from age 4 to 8 weeks on the subsequent course of hypertension and renal disease in the WKY and SHR. This second experiment was designed to be the "mirror image" of previous studies using a renin-angiotensin system inhibitor to suppress hypertension. In other words, we examined if treatment of normotensive (WKY) or hypertensive (SHR) rats during the "critical period" in the development of hypertension would result in the development of hypertension in the case of the WKY, or a worsening of the hypertension in the SHR.

We found that the rats treated with Ang II showed a small but consistent increase in blood pressure compared with the rats administered saline during the same period, and that this difference was accentuated after L-NAME treatment. Moreover, we observed that the SHR exposed to Ang II had the highest values of BUN, creatinine and urine protein among the 4 groups, while the vascular injury scores and kidney weights were significantly increased in these rats compared to the SHR Ang II (–) rats. Taken together, these results suggest that in the WKY/SHR model, developmental activity of the renin-angiotensin system is important in determining the blood pressure in later life, as well as the subsequent response to L-NAME treatment.

Several mechanisms could be involved in the sustained effects on hypertension. One potential mechanism is that the development of hypertension could cause a compensatory increase in vascular hypertrophy, leading to increased peripheral vascular resistance, which in turn could initiate a vicious cycle causing further increases in blood pressure. Consistent with this hypothesis is the fact that the values of aortic media thickness, an index of vascular thickening, showed a trend similar to that of the final blood pressures in both experiments.

Another important observation is the changes in the renin-angiotensin system. In this study, the levels of PRA and other components of the renin-angiotensin-aldosterone system were elevated following L-NAME treatment as reported previously (*16*). In the first experiment, these increases were inhibited by the candesartan (transient) treatment, but not by transient treatment with nicardipine or hydralazine. In the second experiment, increases in the levels of several components of the renin-angiotensin-system were observed in the rats exposed to Ang II.

Since the renin-angiotensin system is involved in the development of vascular hypertrophy, we have developed a hypothesis (the "reno-vascular amplifier" hypothesis) to explain all these observations. As shown in Fig. 10, an increase in the blood pressure causes compensatory vascular hypertrophy and narrowing of the vascular lumen, resulting in renal ischemia and hypoperfusion of the juxtaglomerular apparatus (JGA). This leads to increased renin synthesis, which worsens the hypertension and vascular hypertrophy, initiating a vicious cycle. Treatment with an ARB attenuates both the blood pressure increase and the vascular hypertrophy, and suppresses the vicious cycle, whereas Ang II treatment accelerates this cycle.

Although this hypothesis could explain the results of this study, other potential mechanisms may also be considered, such as changes in sympathetic nerve activity (*17*), or changes in JGA functions at the cellular level. It is also possible that the primary mechanism may be different in other models of

hypertension. Further studies are required to confirm the importance of this putative "reno-vascular amplifier" mechanism in the development of hypertension in different models of hypertension.

An interesting finding of this study was that the rats in both the candesartan (transient) and (sustained) groups showed a clear resistance to the L-NAME–induced increases in oxidative stress. Increases in oxidative stress have been considered to cause end-organ damage which can occur not only in hypertension, but also in other related diseases, including diabetes, hyperlipidemia, and obesity (*18–20*). At present, it is unclear whether the changes in oxidative stress levels are a cause or a consequence of the hypertension and renal damage, and further studies are required to examine this issue in greater detail.

It should be noted that the rats in the candesartan (sustained) group had a tendency toward reduced body weights compared to the animals in the other groups. Because of these effects on the body weight, the abilities of candesartan to attenuate heart weights and media thickness were statistically significant in the absence of body weight correction, but lost statistical significance when the values were corrected for body weight. Recently, Kouyama *et al*. reported that AT1a knock-out mice exhibited attenuation of diet-induced weight gain. The mechanism appears to involve a modulation of adipocytokine production by the AT1a receptor, resulting in reduced adiposity through increased energy expenditure, without a change in food intake (*21*). However, it is unlikely that the observed decreases in the heart weights and aortic media thickness in the present study were caused by reduced food intake and malnutrition, because the changes in body weights could be explained by reduced adipose tissue.

The results of this study may have important clinical implications. Although the pathophysiology of hypertension in the SHR may not be identical to that in human essential hypertension, our study supports the view that inhibition of the renin-angiotensin system before the appearance of hypertension (*i.e.*, at the pre-hypertension stage) could have long-term effects on the later development of hypertension and hypertensive end-organ damage. This would provide a rationale for the treatment of hypertension at an earlier stage in order to attenuate the severity of hypertension and hypertensive renal injury in susceptible individuals. In this connection, a recent clinical study (the TROPHY study) has suggested that treatment of prehypertensive patients is feasible and may reduce the risk of subsequent development of hypertension (*15*). Focusing on interventions before the appearance of hypertension or renal damage may be important for the prevention of hypertension and renal injury at a later time point.

In summary, the results of this study suggest that transient treatment with an ARB during a "critical period" in hypertension development causes suppression of the renin-angiotensin-aldosterone system, and provides long-lasting protection against the later development of acquired hypertension, renal injury, and oxidative stress in the L-NAME model. Exposure to Ang II has the opposite effect, enhancing the activity of the renin-angiotensin-aldosterone system, and predisposing rats to increased blood pressure, renal injury, and oxidative stress. These results support the hypothesis that the activity of the renin-angiotensin during this stage in hypertension development may play an important role in determining the susceptibility of individuals to hypertension, renal injury, and oxidative stress in later life.

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