

## AT<sub>1</sub> receptor antagonist combats oxidative stress and restores nitric oxide signaling in the SHR

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**AT<sub>1</sub> receptor antagonist combats oxidative stress and restores nitric oxide signaling in the SHR.** The tubuloglomerular feedback (TGF) responses of the spontaneously hypertensive rat (SHR) are under exaggerated regulation by angiotensin II (Ang II) type 1 receptors (AT<sub>1</sub>-R). Since AT<sub>1</sub>-Rs enhance oxygen radical (O<sub>2</sub><sup>-</sup>) generation, we tested the hypothesis that the exaggerated TGF was due to a diminished blunting by macula densa (MD)-derived nitric oxide (NO) because of excessive AT<sub>1</sub>-R-dependent generation of O<sub>2</sub><sup>-</sup>. Groups of SHR and control Wistar-Kyoto (WKY) rats received vehicle (Veh), the AT<sub>1</sub>-R antagonist candesartan (Cand; 3 mg · kg<sup>-1</sup> · day<sup>-1</sup>), or nonspecific therapy with hydralazine + hydrochlorothiazide + reserpine (HHR) for two weeks. Compared with WKY rats, the elevated mean arterial pressure of SHR (WKY 125 ± 2 vs. SHR 163 to 779 mm Hg, *P* < 0.001) was reduced (*P* < 0.001) similarly in SHR by Cand and HHR (121 ± 5 and 116 ± 5 mm Hg, *P* = NS). The SHR had an increased maximal TGF response (change in stop flow pressure during luminal perfusion of fluid: SHR 11.2 ± 0.5 vs. WKY 8.3 ± 0.4 mm Hg, *P* < 0.01) and a reduced TGF response to blockade of neuronal NO synthase (nNOS) in the MD with luminal 7-nitroindazole (7-NI: ΔTGF in WKY 2.8 ± 0.4 vs. SHR 1.1 ± 0.6 mm Hg, *P* < 0.05). Although the elevated TGF responses of SHR were normalized by both HHR and Cand, only Cand restored a normal TGF response to luminal perfusion of the MD with 7-NI (ΔTGF with 7-NI in SHR: Veh + 1.8 ± 0.4 vs. Cand + 3.4 ± 0.5 mm Hg, *P* < 0.05). To abrogate the local effects of O<sub>2</sub><sup>-</sup>, tempol (a membrane-permeable superoxide dismutase mimetic) was perfused into the efferent arteriole. During tempol, SHR given vehicle or HHR had a much increased response to blockade of nNOS with 7-NI (ΔTGF in SHR with 7-NI during tempol: Veh 6.3 ± 1.0 and HHR 4.5 ± 0.8 mm Hg, *P* < 0.01 vs. no tempol for both), implying that the effects of NO had been prevented because of excessive O<sub>2</sub><sup>-</sup>. In contrast, the TGF response to 7-NI in SHR given Cand was unaffected by tempol (ΔTGF with 7-NI during tempol: 2.9 ± 0.9, *P* = NS, compared with no tempol). In conclusion, TGF responses of SHR are exaggerated because of the effects of hypertension and AT<sub>1</sub>-R. AT<sub>1</sub>-R blockade specifically diminishes oxidative stress and restores NO signaling in the juxtaglomerular apparatus of the SHR.

**Key words:** oxygen radicals, reactive oxygen species, tubuloglomerular feedback, hypertension, glomerular filtration rate, tempol, 7-nitroindazole, candesartan.

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Hypertension is accompanied by oxidative stress of large conduit blood vessels and certain organs, including the kidney [1]. Presently, neither the cause of the oxidative stress nor its role in the regulation of the tone of the major renal resistance vessels is clear. Studies with a cell membrane-permeable form of superoxide dismutase (SOD) or with the nitroxide SOD mimetic tempol have implicated oxygen radicals (O<sub>2</sub><sup>-</sup>) in the hypertension [2, 3], renal vasoconstriction [4], and enhanced tubuloglomerular feedback (TGF)-dependent tone of the renal afferent arteriole [5] of the spontaneously hypertensive rat (SHR) model.

Angiotensin II (Ang II) induces oxidative stress, as indicated by enhanced levels of 8-iso prostaglandin F<sub>2α</sub> (8-iso-PGF<sub>2α</sub>) [6]. There is experimental evidence of excessive Ang II type 1 receptor (AT<sub>1</sub>-R)-dependent vasoconstriction specifically in the kidney of the SHRs. Thus, although the circulating levels of Ang II and intrarenal levels of Ang I, Ang II, and Ang 1–7 are normal or low in the SHR [7], there is an exaggerated blood pressure (BP) response to renin-angiotensin-aldosterone system (RAAS) blockade [8] and an exaggerated renal vasoconstrictor response to Ang II [9, 10] that cannot be ascribed to enhanced renal or glomerular expression of AT<sub>1</sub> or AT<sub>2</sub> receptors [9, 11, 12]. The enhanced response to Ang II is specific for the kidney [10] and may underlie an enhanced hypertensinogenic action of Ang II in the SHR [13].

Nitric oxide (NO) generated from a neuronal or type 1 constitutive nitric oxide synthase (nNOS) that is heavily expressed in macula densa (MD) cells is activated during MD solute reabsorption. It normally blunts the expression of the TGF response [14]. We found evidence that production of O<sub>2</sub><sup>-</sup> in the juxtaglomerular apparatus (JGA) opposes the normal buffering of TGF by MD-derived NO [15]. This effect of O<sub>2</sub><sup>-</sup> is exaggerated in the JGA of the SHR [5]. The functional effects of O<sub>2</sub><sup>-</sup> could be corrected by microperfusion of tempol, which is a membrane-permeable nitroxide SOD mimetic [16, 17] into the efferent arteriole (EA) that supplies the peritubular capillaries and the interstitium around the MD region.

Oxidative stress is enhanced by Ang II acting on AT<sub>1</sub>

receptors [18–20]. Recent studies have shown that short-term AT<sub>1</sub>-R blockade can restore a normal TGF response in the SHR [21]. Therefore, the present studies were designed to test the hypothesis that AT<sub>1</sub>-R-dependent oxidative stress in the JGA of the SHR model eclipses the blunting of the TGF response by MD-derived NO. These experiments utilized groups of SHR and control Wistar-Kyoto (WKY) normotensive rats treated for two weeks with a vehicle or with a long-acting AT<sub>1</sub>-R antagonist, candesartan. Additional groups received equi-antihypertensive therapy with hydralazine, hydrochlorothiazide, and reserpine (HHR) that was designed to lower BP similarly to candesartan but leave intact the AT<sub>1</sub>-R and stimulate the RAAS [22]. The effects of MD-derived NO were deduced from the responses to luminal microperfusions of the relatively nNOS-specific antagonist 7-nitroindazole (7-NI) [23]. The effects of oxidative stress mediated by O<sub>2</sub><sup>-</sup> in preventing the blunting of TGF by MD-derived NO were deduced from the effects of microperfusion of tempol into the EA supplying the test nephron on the TGF response to luminal microperfusion of 7-NI. These experiments obviate the confounding effects produced by systemic administration of these antagonists and directly target the JGA.

## METHODS

Studies were undertaken on male SHRs and WKY rats whose initial weights were 200 to 250 g (obtained from Harlan-Sprague-Dawley, Madison, WI, USA). They were maintained on a standard rat chow (Purina, St. Louis, MO, USA) with a sodium content of 0.3 g<sup>-1</sup> · 100 g<sup>-1</sup> for one to two weeks before study. They were allowed free access to food and water until the day of study.

### Clearance and TGF measurements

Groups of SHRs and age-matched WKY rats were prepared for *in vivo* clearance, micropuncture, microperfusion, and TGF studies as described in detail previously [24]. In brief, animals were anesthetized with thiobarbital (Inactin, 100 mg · kg<sup>-1</sup> intraperitoneally; Research Biochemicals, Inc., Natick, MA, USA). A catheter was placed in a jugular vein for fluid infusion and in a femoral artery for the recording of mean arterial pressure (MAP) from the electrically damped output of a pressure transducer (Statham, Gould Inc., Oxnard, CA, USA). A tracheotomy tube was inserted. The animals were allowed to breathe spontaneously. A catheter was placed in the bladder to collect urine from both kidneys. The left kidney was exposed by a flank incision, cleaned of connective tissue, and stabilized in a Lucite cup. It was bathed in 0.154 mol/L NaCl maintained at 37°C. After completion of surgery, rats were infused with a solution of 0.154 mol/L NaCl and 1% albumin at 1.5 mL · h<sup>-1</sup> to maintain a

euvolemic state [24]. Micropuncture studies were begun after 60 minutes for stabilization.

Clearance studies were undertaken during the micropuncture measurements. [<sup>3</sup>H]-inulin (0.1 μCi · mL<sup>-1</sup>; ICN Biochemicals, Costa Mesa, CA, USA) was added to the intravenous fluid infusion. Urine was obtained from the bladder, and blood was sampled at the midpoint. The micropuncture period lasted 60 to 90 minutes, during which a renal clearance was undertaken with a blood sample drawn at the midpoint. Values of MAP and heart rate (HR) were obtained over 15-minute intervals during this period and were averaged.

For orthograde microperfusion of the loop of Henle (LH), a micropipette (8 μm OD) containing artificial tubular fluid (ATF) stained with FD&C dye was inserted into a late proximal tubule [24]. Injections of the colored ATF identified the nephron and the direction of flow. An immobile bone wax block was inserted into this micropuncture site via a micropipette (10 to 15 μm) connected to a hydraulic drive (Trent Wells, Inc., La Jolla, CA, USA) to halt tubular fluid flow. A perfusion micropipette (6 to 8 μm) containing ATF with test compounds or vehicle was inserted into the proximal tubule downstream from the wax block and connected to a nanoliter perfusion pump (WPI, Sarasota, FL, USA). A pressure micropipette (1 to 2 μm) was inserted into the proximal tubule upstream from the wax block to measure proximal stop flow pressure (PSF). The pressure was recorded by a servo-null instrument (Instruments for Physiology and Medicine, La Jolla, CA, USA). Changes in PSF are an index of changes in glomerular capillary hydraulic pressure. Measurements of PSF were made in each nephron during zero loop perfusion and during perfusion with ATF at 40 nL · min<sup>-1</sup>. This produces a maximal TGF response, defined as the difference between PSF values recorded during perfusion of the loop with ATF at 0 and 40 nL · min<sup>-1</sup>. Maximal rat TGF responses were determined in one to three nephrons.

### Series 1

Measurements were made during perfusion of the LH with ATF + vehicle and were contrasted with ATF + 7-NI (10<sup>-4</sup> mol/L). We had found previously that this dose of 7-NI was maximally effective and produced a rapid and reversible increase in TGF responses [25].

### Series 2

In separate groups of rats, the EA supplying the test nephron was perfused at 15 nL · min<sup>-1</sup> with artificial plasma (AP) + tempol (10<sup>-4</sup> mol/L) [24] 2,2,6,6-tetramethyl-1-piperidinyloxy [2, 16, 17] and stained with FD&C green dye. After five minutes, with the EA microperfusion continuing, the TGF was tested with luminal perfusions of ATF + vehicle and ATF + 7-NI at 0 and 40 nL · min<sup>-1</sup>. The order of luminal perfusions

**Table 1.** Body weights, mean arterial pressure (MAP), heart rate (HR), urine flow (UV) and glomerular filtration rate (GFR)

Group	N	Body wt g	MAP mmHg	HR beats·min <sup>-1</sup>	UV μL·min <sup>-1</sup> ·100 g <sup>-1</sup>	GFR mL·min <sup>-1</sup> ·100 g <sup>-1</sup>
1. WKY, Veh	6	358 ± 17	125 ± 2	368 ± 5	1.7 ± 0.3	0.73 ± 0.06
2. WKY, HHR	5	342 ± 12	98 ± 5	355 ± 8	1.8 ± 0.3	0.70 ± 0.07
<i>P</i> values vs. #1		NS	<0.01	NS	NS	NS
3. WKY, Cand	6	358 ± 12	103 ± 2	363 ± 5	2.1 ± 0.4	0.79 ± 0.08
<i>P</i> values vs. #1		NS	<0.01	NS	NS	NS
4. SHR, Veh	6	296 ± 10	163 ± 9	370 ± 11	2.0 ± 0.4	0.51 ± 0.08
<i>P</i> values vs. #1		<0.01	<0.01	NS	NS	<0.05
5. SHR, HHR	5	307 ± 9	116 ± 5	375 ± 12	1.7 ± 0.3	0.54 ± 0.10
<i>P</i> values vs. #4		NS	<0.01	NS	NS	NS
6. SHR, Cand	6	285 ± 10	121 ± 5	362 ± 4	1.9 ± 0.3	0.79 ± 0.09
<i>P</i> values vs. #4		NS	<0.01	NS	NS	<0.05

Values are mean ± SEM (*N* = number of rats studied) for rats of series 1. Abbreviations are in the **Appendix**.

was randomized. We have shown previously that EA perfusion with AP at 15 to 20 nL·min<sup>-1</sup> does not perturb the TGF responses [24]. Only nephrons in which the surrounding interstitium was tinted with the colored AP perfusion solution were included in the study.

### Protocols

For 14 to 18 days, groups (*N* = 6 to 8) of SHRs and WKY rats received a daily gavage with vehicle (Veh), candesartan (Cand; 3 mg·kg<sup>-1</sup>·day<sup>-1</sup>) or hydralazine + hydrochlorothiazide + reserpine (HHR; 30 + 10 + 0.2 mg·kg<sup>-1</sup>·day<sup>-1</sup>). Drugs were withheld on the day of study.

### Drugs

The soluble salt of 7-NI was obtained from Tocris Cookson, Inc. (St. Louis, MO, USA). Tempol was obtained from Aldrich Chemical Co. (Milwaukee, WI, USA). Other chemicals were obtained from Sigma (St. Louis, MO, USA).

### Statistical methods

Values are reported as mean ± SEM. A repeated-measures analysis of variance (ANOVA) was applied to data of SHRs and WKY rats administered vehicle, candesartan, or HHR. Where appropriate, a post hoc Dunnett's *t* test was applied thereafter. Values are taken as statistically significant if *P* < 0.05.

### RESULTS

Data for whole kidney function in rats of series 1 are shown in Table 1. Data for series 2 were strictly similar, but clearance measurements of glomerular filtration rate (GFR) were made only in series 1. The body weights for WKY rats at the time of study were significantly higher than SHR, reflecting their better rate of growth. The MAP was increased in the vehicle-administered SHR compared with equivalent WKY. Both Cand and HHR reduced the MAP of the SHRs to levels that were

not significantly different between the two treatments or from the values of WKY rats given vehicle. Therefore, both treatments effectively normalized the MAP of the SHR. These drugs also reduced the MAP of WKY rats. The HRs and rates of urine flow were similar between groups. The mean values for the GFR were significantly (*P* < 0.05) lower by 31% in vehicle-treated SHRs compared with WKY rats. The GFR of SHRs was not modified by treatment with HHR but was increased significantly (*P* < 0.05) by Cand to levels of vehicle-treated WKY rats.

Tubuloglomerular feedback parameters are shown in Table 2. The PSF at zero LH perfusion was elevated (*P* < 0.05) by approximately 2 mm Hg in hypertensive SHR given vehicle compared with equivalent WKY rats. This was anticipated from a prior study that had shown that the PSF at zero loop perfusion is dependent on the BP [26]. The maximal TGF responses during loop perfusion with ATF + vehicle were enhanced significantly (*P* < 0.001) by 35% in vehicle-administered SHR compared with comparable WKY. Both Cand and HHR reduced the exaggerated TGF responses of SHR significantly (*P* < 0.001) to values comparable to the vehicle-treated WKY, whereas these treatments did not modify the TGF responses of the WKY.

The role of MD-derived NO in blunting TGF responses was assessed from the increase in TGF during blockade of nNOS by luminal microperfusion of 7-NI (Table 2). Whereas WKY had a consistent increase in maximal TGF responses with 7-NI of 2.8 ± 0.4 mm Hg, there was a significantly (*P* < 0.05) blunted rise of 1.1 ± 0.6 mm Hg in vehicle-treated SHR. The increase in TGF with 7-NI was not increased significantly above vehicle treatment in WKY rats given HHR (2.8 ± 0.6 mm Hg) or Cand (2.4 ± 0.4 mm Hg). SHRs receiving HHR had only a modest increase in TGF with 7-NI of 1.8 ± 0.4 mm Hg that was not significantly different from the increase in vehicle-administered SHRs of 1.1 ± 0.6 mm Hg. However, SHRs receiving Cand had an in-

**Table 2.** Proximal stop flow pressure (PSF) during luminal microperfusion of artificial tubular fluid (ATF) containing vehicle (Veh) or 7-nitroindazole (7-NI)

Group	Added to ATF	PSF (mm Hg) during LH microperfusion at (nL·min <sup>-1</sup> )		
		0	40	0-40
WKY				
1. WKY, Veh (N=6)				
	Veh	38.5 ± 0.4	30.2 ± 0.8	8.3 ± 0.4
	7-NI	38.2 ± 0.4	27.1 ± 0.6	11.2 ± 0.4
	Δ	+0.1 ± 0.3	-2.9 ± 0.9	+2.8 ± 0.4
	P value	NS	<0.01	<0.001
2. WKY, HHR (N=5)				
	Veh	37.5 ± 1.0	29.0 ± 1.0	8.4 ± 0.8
	7-NI	38.0 ± 1.1	26.6 ± 0.7	11.2 ± 0.8
	Δ	+0.4 ± 0.4	-2.4 ± 0.8	+2.8 ± 0.6
	P value	NS	<0.001	<0.001
3. WKY, Cand (N=6)				
	Veh	36.8 ± 0.5	28.7 ± 0.5	7.8 ± 0.3
	7-NI	36.8 ± 0.6	26.5 ± 0.8	10.3 ± 0.5
	Δ	0.0 ± 0.3	-2.5 ± 0.7	+2.4 ± 0.4
	P value	NS	<0.001	<0.001
SHR				
4. SHR, Veh (N=6)				
	Veh	40.0 ± 0.8	29.2 ± 0.5	10.9 ± 0.7
	7-NI	40.0 ± 0.7	27.5 ± 0.7	12.0 ± 0.7
	Δ	-0.2 ± 0.3	-1.7 ± 0.7	+1.1 ± 0.6
	P value	NS	<0.05	NS
5. SHR, HHR (N=5)				
	Veh	37.2 ± 0.7	29.7 ± 0.6	7.3 ± 0.7
	7-NI	37.2 ± 0.8	28.1 ± 0.7	9.1 ± 0.6
	Δ	0.0 ± 0.3	-1.5 ± 0.4	+1.8 ± 0.4
	P value	NS	<0.001	<0.01
6. SHR, Cand (N=6)				
	Veh	37.3 ± 0.8	29.0 ± 0.5	7.8 ± 0.5
	7-NI	37.2 ± 0.8	26.0 ± 0.4	11.4 ± 0.7
	Δ	+0.1 ± 0.3	-3.0 ± 0.7	+3.4 ± 0.5
	P value	NS	<0.001	<0.001

Data are mean ± SEM (N = number of rats studied). ATF is artificial tubular fluid perfused orthograde through the loop of Henle.

crease in TGF with luminal 7-NI of 3.4 ± 0.5 mm Hg that was significantly ( $P < 0.05$ ) greater than in SHRs receiving HHR and significantly ( $P < 0.01$ ) greater than in SHRs receiving vehicle. Cand effectively normalized the TGF response to 7-NI; during candesartan, the response of SHRs to 7-NI was not significantly different from vehicle-treated WKY rats. We conclude that SHRs have a diminished blunting of TGF by MD-derived NO that is restored in full by candesartan, but not by HHR.

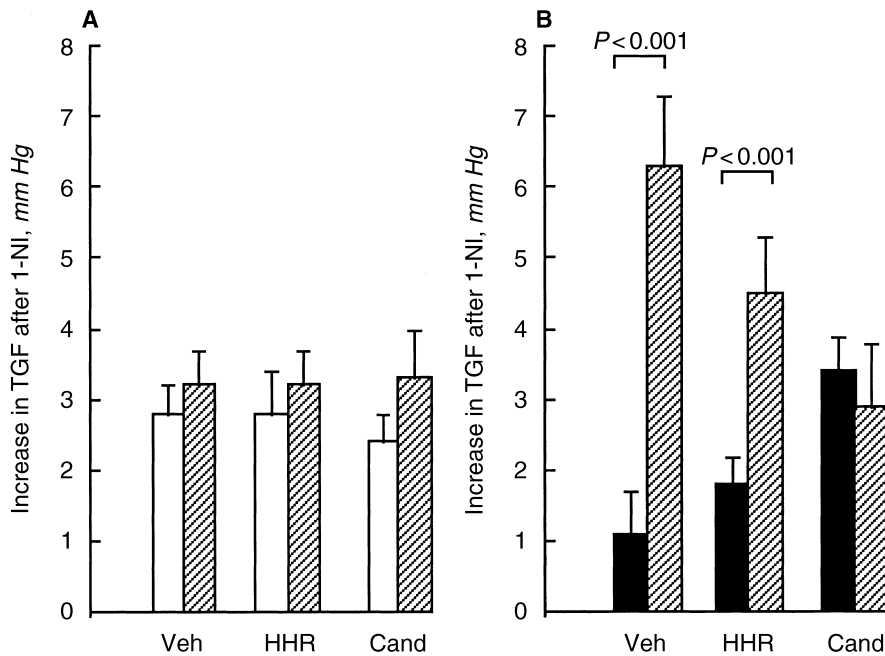
Table 3 details the TGF responses to loop perfusion of ATF + 7-NI compared with ATF + vehicle during EA perfusion of tempol. As described previously, tempol blunted TGF responses to ATF + vehicle more in SHR than WKY rat nephrons [5]. We interpret any effect of tempol to increase the effectiveness of 7-NI in enhancing TGF to represent an effect of short-term abrogation of oxidative stress to restore NO signaling in the JGA. As shown in Figure 1, the increases in TGF produced by luminal 7-NI in groups of WKY administered vehicle,

**Table 3.** Proximal stop flow pressure (PSF) to luminal perfusion of artificial tubular fluid (ATF) containing vehicle (Veh) or 7-nitroindazole (7-NI) during microperfusion of tempol into the efferent arteriole

Group	Added to ATF	PSF (mm Hg) during LH microperfusion at (nL·min <sup>-1</sup> )		
		0	40	0-40
WKY				
1. WKY, Veh (N=6)				
	Veh	39.4 ± 0.5	32.6 ± 0.3	7.0 ± 0.3
	7-NI	39.5 ± 0.5	27.4 ± 0.9	10.2 ± 0.5
	Δ	+0.1 ± 0.3	-5.3 ± 0.9	+3.2 ± 0.5
	P value	—	0.001	0.001
2. WKY, HHR (N=6)				
	Veh	38.2 ± 0.5	31.8 ± 0.3	6.4 ± 0.4
	7-NI	38.6 ± 0.6	29.1 ± 0.4	9.6 ± 0.4
	Δ	+0.5 ± 0.3	-2.6 ± 0.5	+3.2 ± 0.5
	P value	NS	0.001	0.001
3. WKY, Cand (N=8)				
	Veh	38.9 ± 0.4	32.1 ± 0.3	6.9 ± 0.5
	7-NI	38.7 ± 0.3	29.2 ± 0.4	9.6 ± 0.4
	Δ	-0.2 ± 0.3	-2.9 ± 0.6	+3.2 ± 0.7
	P value	NS	0.001	0.001
SHR				
4. SHR, Veh (N=7)				
	Veh	41.1 ± 0.4	33.4 ± 0.5	7.5 ± 0.5
	7-NI	40.9 ± 0.5	27.0 ± 0.6	13.8 ± 0.4
	Δ	-0.2 ± 0.3	-6.5 ± 0.7	+6.3 ± 1.0
	P value	NS	0.001	0.001
5. SHR, HHR (N=7)				
	Veh	39.7 ± 0.7	32.9 ± 0.5	6.9 ± 0.5
	7-NI	39.2 ± 1.1	27.8 ± 0.5	11.5 ± 1.0
	Δ	-0.5 ± 0.3	-3.1 ± 0.5	+4.5 ± 0.8
	P value	NS	0.001	0.001
6. SHR, Cand (N=9)				
	Veh	37.2 ± 0.9	29.7 ± 0.5	7.9 ± 0.5
	7-NI	37.9 ± 0.9	27.2 ± 0.4	10.7 ± 0.4
	Δ	+0.7 ± 0.5	-2.6 ± 0.5	+2.9 ± 0.9
	P value	NS	0.001	0.001

Data are mean ± SEM values (N = number of rats studied). ATF is the artificial tubular fluid perfused orthograde and through the loop of Henle.

HHR, or Cand were not significantly different from those seen in the absence of tempol. In contrast, in vehicle-treated SHR microperfused with tempol, there was a robust enhancement of TGF by 7-NI of 6.3 ± 1.0 mm Hg that was sixfold greater ( $P < 0.001$ ) than the response to 7-NI of 1.1 ± 0.6 mm Hg seen in the absence of tempol. During the administration of HHR, SHR nephrons microperfused with tempol had an increase in TGF with luminal 7-NI of 4.5 ± 0.8 mm Hg that was threefold greater ( $P < 0.01$ ) than the increase of 1.8 ± 0.4 mm Hg seen without tempol. This increase in TGF with 7-NI during tempol in SHRs treated with HHR was not significantly different from SHRs treated with vehicle. In contrast, during the administration of Cand, SHR nephrons microperfused with tempol had an increase in TGF with 7-NI of 2.9 ± 0.9 mm Hg that was not significantly different from the increase of 3.4 ± 0.5 mm Hg seen without tempol. We conclude that the effects of O<sub>2</sub><sup>-</sup> to



**Fig. 1.** Mean  $\pm$  SEM values for absolute increases in maximal tubuloglomerular feedback (TGF) responses during luminal perfusion with artificial tubular fluid (ATF) + 7-nitroindazole (7-NI;  $10^{-4}$  mol/L) compared with ATF + vehicle. Data are compared in nephrons studied without ( $\square$ ; A or  $\blacksquare$ ; B) or during ( $\text{hatched}$ ) microperfusion of tempol ( $10^{-4}$  mol/L) in artificial plasma (AP) into the efferent arteriole (EA). Groups of WKY rats (A) or SHRs (B) were administered with vehicle (Veh), hydralazine + hydrochlorothiazide + reserpine (HHR), or candesartan (Cand) for two weeks prior to testing.

prevent blunting of TGF responses by NO were exaggerated in the SHRs. This effect was prevented in full only by candesartan.

## DISCUSSION

Brannstrom, Morsing, and Arendshorst reported that a single intravenous bolus of candesartan normalizes the exaggerated TGF responses of young SHR rat nephrons [21]. This was confirmed in the present study, which utilized adult SHRs that were administered candesartan over two weeks. In the prior study, candesartan normalized both the enhanced TGF-induced changes in PSF and SNGFR, and the responsiveness and the set point (sensitivity) of the TGF response. Our results extend the prior evidence for an exaggerated role for AT<sub>1</sub>-R in the kidneys of the SHR to the JGA [8–13]. In confirmation of the findings of Brannstrom et al with short-term candesartan [21], we found that this drug given over two weeks did not perturb the TGF response of normotensive WKY rats. Clearly, this was an effective dose of candesartan in WKY since it reduced the MAP in the current study (Table 1), and the dose used by Brannstrom et al blocked the renal and systemic effects of infused Ang II [21]. These results differ from the short-term effects of an angiotensin-converting enzyme inhibitor [27–29] or an Ang II antagonist [29, 30], which impairs TGF responses in normotensive rats. Moreover, mice with targeted disruption of the AT<sub>1</sub> receptor have no TGF responses [31]. In the present study, candesartan failed to blunt the TGF responses despite a fall in MAP, which itself normally depresses TGF [26]. Since the effects of Ang II are strictly

dependent on the extracellular fluid volume, it may be that subtle differences in the experimental conditions between these studies underlie these divergent responses to angiotensin blockade.

We showed previously that whereas microperfusion of 7-NI into WKY rat nephrons to block nNOS increases TGF significantly, it has little effect in SHR nephrons [25]. We postulated that this failure of a functional response to nNOS blockade in SHR nephrons might be a secondary consequence of oxidative stress, since microperfusion of tempol into the JGA caused a very modest blunting of TGF of normotensive rats [15], but a significantly enhanced blunting of the TGF in SHR [5]. The present studies confirm that microperfusion of tempol reduces the TGF of WKY modestly by 16%, yet reduces the TGF more prominently in SHR by 33% (Tables 2 and 3). A new finding in the present study is that prolonged blockade of AT<sub>1</sub> receptors in the SHR restores a normal functional response to nNOS blockade (Fig. 1).

The administration of hydralazine, hydrochlorothiazide, and reserpine increases plasma renin activity in the rat [22]. These drugs were as effective as candesartan in reducing the BP and normalizing the TGF responses in the SHR. Nevertheless, there were important differences in the responses of the SHR kidney to the two types of therapy. First, only AT<sub>1</sub>-R blockade normalized the reduced GFR. Second, only AT<sub>1</sub>-R blockade normalized the TGF responses to nNOS blockade with 7-NI. We conclude that prolonged AT<sub>1</sub>-R blockade has specific effects in the JGA of the SHR model of hypertension above those of reducing the BP.

Mitchell et al have shown that tempol acts as an SOD

mimetic and enhances H<sub>2</sub>O<sub>2</sub> metabolism to O<sub>2</sub> and H<sub>2</sub>O [16, 32, 33]. It is highly permeable to cell membranes and is not toxic. It does not bind directly to nor generate NO [33]. It is a validated spin-trap for O<sub>2</sub><sup>-</sup> in electron paramagnetic resonance studies [32]. Tempol protects cells and tissues against oxidative stress [34]. It reduces the elevated BP, the excretion of 8-iso-PGF<sub>2α</sub>, and the renal vascular resistance of the SHR [2, 4]. Microperfusion of tempol into the EA enhances the sensitivity and responsiveness of NO donor compounds in blunting TGF when applied to the lumen of the MD [5]. Therefore, tempol was selected for these studies to evaluate the role of excessive O<sub>2</sub><sup>-</sup> generation in the SHR.

Macula densa-derived NO establishes the set point for TGF during long-term adaptation to changes in proximal reabsorption [35]. Prolonged inhibition of nNOS with oral 7-NI in normal rats first enhances TGF and later induced hypertension [36]. These studies establish an important long-term role for MD nNOS to modulate TGF. However, an intact nNOS is not an absolute requirement for TGF responses since TGF is normal in nNOS knockout mice (abstract; Schnermann et al, *J Am Soc Nephrol* 10:387A, 1999). The exaggerated blunting of TGF by tempol in SHR confirms a prior study [5]. There is a diminished buffering of TGF by NO derived from nNOS in the JGA of the SHR since, as in previous studies [25, 37], there was no significant response of TGF to blockade of NOS in SHR nephrons. The present study provides evidence that this diminished role for NO in the JGA of the SHR can be ascribed to enhanced oxidative stress because SHR nephrons had an exaggerated response to the nNOS blockade during local microperfusion of tempol. This is consistent with our previous finding that the mRNA and protein expressions for nNOS in the renal cortex and nNOS immunoreactivity in the MD are enhanced in the SHR kidney [25]. Apparently, the overexpressed nNOS is functional in the SHR only after abrogation of the effects of oxidative stress. Peroxynitrite (ONOO<sup>-</sup>), which is formed by the reaction of NO and O<sub>2</sub><sup>-</sup>, can nitrate tyrosine residues in proteins. Bosse and Bachmann showed intense nitrotyrosine immunoreactivity in the Ang II-stimulated postclip kidney of Goldblatt hypertensive rats [20]. Immunoreactivity was expressed strongly in the interstitial spaces between the MD and the afferent arteriole. This suggests that O<sub>2</sub><sup>-</sup>, likely generated from nicotinamide adenine dinucleotide phosphate (NADPH) oxidase that is expressed in interstitial fibroblasts and mesangial cells [19] and is stimulated by Ang II [18], forms a barrier in the hypertensive kidney that limits the effects of MD derived NO on the afferent arteriole.

What can be concluded from these data concerning the relative importance of hypertension and AT<sub>1</sub>-R in blunting the function of NO in the JGA of the SHR? Both HHR and Cand normalized the BP and the TGF

responses. This points to an important role for hypertension per se in the exaggerated TGF responses of the SHR [26]. However, Cand restored in full a normal TGF response to the blockade of nNOS, whereas HHR was not effective. Of particular interest were differences in the effects of tempol on the responses to 7-NI. SHR treated with HHR had an enhanced TGF responses to 7-NI during local microperfusion of tempol that was quite similar to SHR treated with vehicle. This suggests that HHR did little to abrogate oxidative stress, despite its effects in lowering BP (Fig. 1), possibly because any beneficial effects of a two-week reduction in BP produced by HHR were offset by an enhanced release of renin and activation of AT<sub>1</sub> receptors [22]. In contrast, SHR treated with candesartan had a TGF response to 7-NI that was unaffected by tempol. This is consistent with the hypothesis that AT<sub>1</sub>-R blockade had effectively prevented the oxidative stress that is characteristic of the SHR. Thus, oxidative stress in the JGA of the SHR can be ascribed to prolonged stimulation of AT<sub>1</sub>-R. Blockade of AT<sub>1</sub>-R apparently restores normal oxidative function and NO signaling within the JGA, restores a normal level of glomerular filtration, and reverses hypertension. Further studies are required to test whether the special effect of angiotensin-converting enzyme inhibition in preventing progressive renal damage in hypertension [22] also depends on restoring a normal functional role for NO.

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## APPENDIX

Abbreviations used in this article are: Ang II, angiotensin II; AP, artificial plasma; AT<sub>1</sub>, angiotensin II type 1; AT<sub>1</sub>-R, angiotensin II type 1 receptor; ATF, artificial tubular fluid; Cand, candesartan; EA, efferent arteriole; GFR, glomerular filtration rate; HHR, hydralazine + hydrochlorothiazide + reserpine; HR, heart rate; JGA, juxtaglomerular apparatus; LH, loop of Henle; MAP, mean arterial pressure; MD, macula densa; 7-NI, 7-nitroindazole; NO, nitric oxide, nNOS, neuronal nitric oxide synthase; O<sub>2</sub><sup>-</sup>, oxygen radical; PSF, proximal stop flow pressure; SHR, spontaneously hypertensive rat; SOD, superoxide dismutase; TGF, tubuloglomerular feedback; Veh, vehicle; WKY, Wistar Kyoto rat.

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