Blunted Tubuloglomerular Feedback by Absence of Angiotensin Type 1A Receptor Involves Neuronal NOS

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Abstract—To define the role of angiotensin type 1A (AT₁A) receptor in modulating tubuloglomerular feedback signals and to determine its relationship to neuronal NO synthase (nNOS), the diameter of the afferent arterioles of wild-type and AT₁A receptor–deficient mice was measured by the blood-perfused juxtamedullary nephron technique. The afferent arteriolar diameter of wild-type and AT₁A receptor–deficient mice averaged 16.7±0.6 (n=9) and 16.8±0.7 μm (n=9), respectively. In the wild-type mice, addition of 10 μmol/L acetazolamide to the blood perfusate exerted a biphasic afferent arteriolar constriction, with the initial response and sustained response averaging 47.2±3.8% and 33.9±3.3%, respectively. In AT₁A receptor–deficient mice, the initial response and sustained response averaged 51.6±3.6% and 9.5±1.3%, respectively, and the sustained response was significantly attenuated compared with that of wild-type mice.

Inhibition of nNOS with 10 μmol/L S-methyl-L-thiocitrulline significantly decreased the afferent arteriolar diameter of AT₁A receptor–deficient mice, from 15.1±1.2 to 5.0±0.3 μm (n=7), and the decrease was significantly greater than that observed in wild-type mice (from 15.9±1.2 to 10.6±1.3 μm; n=8). During nNOS inhibition, the initial and sustained afferent arteriolar constrictor responses to acetazolamide in wild-type mice averaged 54.4±6.4% and 44.8±11.3%; respectively, and were similar to those in AT₁A receptor–deficient mice (53.2±6.4% and 59.5±4.4%, respectively). These results suggest that AT₁A receptors enhance tubuloglomerular feedback–mediated afferent arteriolar constriction, at least in part, through reducing the counteracting modulation by nNOS. (Hypertension. 2002;40:934-939.)

Key Words: arterioles • autoregulation • mice • nitric oxide synthase • receptors, angiotensin II

The tubuloglomerular feedback (TGF) mechanism is recognized as the main mechanism responsible for the intrarenal vascular resistance adjustments that occur in response to increases in distal volume and sodium chloride/bicarbonate delivery. Peritubular perfusion of angiotensin II enhances TGF responses, and TGF responses do not occur in angiotensin type 1A (AT₁A) receptor–deficient mice or in ACE-deficient mice. These findings suggest that angiotensin II is a significant constituent of the TGF control system.

Immunohistochemical studies have demonstrated the specific localization of neuronal NO synthase (nNOS) in macula densa cells, which serve as the sensing site of the TGF mechanism by monitoring flow-mediated changes in tubular fluid composition to the distal afferent arteriole, by releasing vasoactive paracrine signals that alter afferent arteriolar resistance. NO produced in response to activation of macula densa nNOS affects the diameter of afferent and efferent arterioles and contributes to the counteracting resetting process of the TGF-mediated autoregulatory response, resulting in a biphasic afferent arteriolar constriction. Thus, macula densa nNOS activity plays an important role in the counteracting modulation of TGF responses.

AT₁ receptors are also present in macula densa cells, and long-term infusion of exogenous angiotensin II decreases the contribution of macula densa nNOS to TGF responses. Because the expressions of the nNOS gene and protein in the macula densa are upregulated in angiotensinogen-gene-knockout mice and AT₁A receptor–deficient mice, the enhancement of TGF responses by angiotensin II may be at least partially owing to decreased activity of macula densa nNOS.

We hypothesized that the physiological presence of AT₁A receptors suppresses macula densa nNOS activity, which exerts counteracting modulation, resulting in biphasic TGF-mediated afferent arteriolar constriction. To test this hypothesis, in the present study, we applied the blood-perfused juxtamedullary nephron technique to the kidneys of wild-type and AT₁A receptor–deficient mice. The TGF response was induced by acute treatment of the kidneys with the carbonic anhydrase inhibitor acetazolamide, which decreases the net proximal tubular reabsorption rate and thus increases volume and salt delivery to macula densa cells. Then, the “initial” and “sustained” constrictor responses of the afferent arterioles to acetazolamide were measured as changes in their...
diameter. The initial response is defined as the maximum decrease in afferent arteriolar diameter after acetazolamide and reflects the early vasoconstrictor autoregulatory response to increases in distal tubular flow. The sustained response is defined as the average change in the final 2 minutes of acetazolamide treatment and involves delayed counteracting vasodilator mechanisms that buffer the magnitude of the response. A recent study demonstrated that nNOS contributes to the sustained response of biphasic afferent arteriolar autoregulatory responses to increasing perfusion pressure. These measurements were also performed during selective inhibition of nNOS with \(5\)-methyl-\(L\)-thiocitrulline (L-SMTC). 7,16

### Methods

#### Measurement of Afferent Arteriolar Diameter

The experiments were performed in accordance with the guidelines for the Care and Use of Laboratory Animals of Keio University School of Medicine. Afferent arteriolar diameter was measured in vitro by using the blood-perfused juxtamedullary nephron technique combined with videomicroscopy, as previously described. 7,8,10,17 Briefly, in each experiment, a male Sprague Dawley rat (Charles River Labs, Yokohama, Japan) was anaesthetized with sodium pentobarbital (50 mg/kg IP). After the dissection was completed, the Tyrode solution (8 g/L NaCl) containing 1% BSA and a mixture of L-amino acids as previously described. 18 The kidney was excised and sectioned longitudinally, retaining the papilla intact with the perfused dorsal two thirds of the organ. The papilla was reflected to expose the pelvic mucosa and tissue covering the inner cortical surface. Overlying tissue was removed to expose the tubules, glomeruli, and related vasculature of the juxtamedullary nephrons. The arterial supply of the exposed microvasculature was isolated by ligating the larger branches of the renal artery with fine suture (nylon black monofilament, 10-0; Vanguard Surgical System).

After the dissection was completed, the Tyrode’s perfusate was replaced with reconstituted rat blood (139 mmol/L acetazolamide (Sigma Chemical Co) to the blood perfusate. Acetazolamide inhibits proximal tubular reabsorption and thus increases distal volume and sodium chloride/bicarbonate delivery. 14,15 We previously demonstrated that this dose of acetazolamide triggers TGF signals that decrease the diameter of the afferent arterioles, 7,19 and the initial and sustained constrictor responses of afferent arterioles to acetazolamide were measured. In addition, the juxtamedullary nephrons visualized in this preparation are known to give rise to long loops of Henle that extend into the papilla before looping back to the distal tubule and past the macula densa. 17 Acute papillectomy interrupts the flow of distal tubular fluid past the macula densa 20 and minimizes TGF-dependent influences on microvascular function. 21 To confirm that the effect of acetazolamide on afferent arteriolar responsiveness resulted from acetazolamide-mediated increases in distal tubular flow, acute papillectomy was performed by cleanly severing the papilla near the corticomedullary junction by a single cut, thereby preventing unnecessary damage to the adjacent tissue. After a 10-minute stabilization period, the diameter of the afferent arterioles was also measured in papillectomized kidneys perfused with the acetazolamide-containing blood.

#### Afferent Arteriolar Responses to nNOS Inhibition

The effect of nNOS blockade on afferent arteriolar diameter was measured in the kidneys of wild-type and \(AT_1\) receptor–deficient mice. Afferent arteriolar diameter was measured before and during exposure to increasing concentrations (0.1, 1, and 10 mmol/L) of the selective nNOS inhibitor L-SMTC (Alexis Corp). After measuring the afferent arteriolar response to L-SMTC, acute papillectomy was performed, and after a 10-minute stabilization period, afferent arteriolar diameter was measured in papillectomized kidneys exposed to 10 mmol/L L-SMTC.

#### Effect of nNOS Inhibition on the Afferent Arteriolar Response to Acetazolamide

The afferent arteriolar response to addition of 10 mmol/L acetazolamide to the blood perfusate was measured during superfusion of the kidneys of wild-type and \(AT_1\) receptor–deficient mice with L-SMTC. After baseline measurement of afferent arteriolar diameter as a control, the superfusate was switched to one containing 10 mmol/L L-SMTC. After a 10-minute stabilization period, the initial and sustained responses of afferent arterioles to acetazolamide were determined during superfusion of L-SMTC. After determination of the afferent arteriolar responses to acetazolamide, acute papillectomy was performed, and the diameter of the afferent arterioles was also measured.

### Statistical Analysis

The changes in afferent arteriolar diameters in response to the experimental conditions were analyzed by 1-way ANOVA for repeated measures combined with the Newman-Keuls post hoc test. The significance of differences in afferent arteriolar diameters between wild-type and \(AT_1\) receptor–deficient mice were determined by the 2-way ANOVA for repeated measures combined with Newman-Keuls post hoc test. A probability value of \(P<0.05\) was considered statistically significant. Data are presented as mean±SE.

### Results

#### Afferent Arteriolar Responses to Acetazolamide

Figure 1 illustrates the afferent arteriolar responses to acetazolamide of \(AT_1\) receptor–deficient mice and wild-type...
The afferent arteriolar diameter of wild-type mice averaged 16.7 ± 0.6 μm (n = 9), and acetazolamide elicited a biphasic afferent arteriolar constrictor response consisting of immediate vasoconstriction (initial response) followed by a modest waning (sustained response). The initial response and sustained response averaged 47.2 ± 3.8% and 33.9 ± 3.3%, respectively. Papillectomy reversed the afferent arteriolar constrictor response to acetazolamide, and the afferent arteriolar diameter after papillectomy averaged 18.7 ± 0.6 μm. In AT1A receptor–deficient mice, afferent arteriolar diameter averaged 16.8 ± 0.7 μm. Acetazolamide elicited a biphasic afferent arteriolar constrictor response consisting of immediate vasoconstriction followed by a significantly greater waning than that observed in wild-type mice. The initial response and sustained response averaged 51.6 ± 3.6% and 9.5 ± 1.3%, respectively. Papillectomy reversed the afferent arteriolar constrictor responses to acetazolamide, and afferent arteriolar diameter after papillectomy averaged 18.9 ± 0.7 μm.

**Afferent Arteriolar Response to nNOS Inhibition**

Figure 2 shows that nNOS inhibition by L-SMTC dose-dependently decreased the diameter of the afferent arterioles of AT1A receptor–deficient mice and wild-type mice. In this series of experiments, the diameter of the afferent arterioles of wild-type mice averaged 15.9 ± 1.2 μm (n = 8) and, at 0.1, 1, and 10 μmol/L concentrations of L-SMTC, significantly decreased to 13.6 ± 1.3, 12.2 ± 1.4, and 10.6 ± 1.3 μm, respectively. Papillectomy prevented the L-SMTC–induced decreases in afferent arteriolar diameter, and after papillectomy, the diameter of the afferent arterioles averaged 17.1 ± 1.5 μm. In AT1A receptor–deficient mice, afferent arteriolar diameter averaged 15.1 ± 1.2 μm (n = 7) and was similar to the diameter in wild-type mice. L-SMTC at concentrations of 0.1, 1, and 10 μmol/L significantly decreased afferent arteriolar diameter to 10.3 ± 0.9, 7.9 ± 0.6, and 5.0 ± 0.3 μm, respectively. The L-SMTC–induced decreases in afferent arteriolar diameter were significantly greater than those observed in wild-type mice and were prevented by papillectomy. Afferent arteriolar diameter after papillectomy averaged 16.8 ± 1.2 μm.

**Effect of nNOS Inhibition on the Afferent Arteriolar Responses to Acetazolamide**

Figure 3 shows the afferent arteriolar responses to acetazolamide treatment during nNOS inhibition with 10 μmol/L L-SMTC. In this series of experiments, the afferent arteriolar diameter of wild-type and AT1A receptor–deficient mice averaged 15.2 ± 1.3 (n = 6) and 15.4 ± 1.0 μm (n = 7), respectively. L-SMTC significantly decreased the afferent arteriolar diameter of wild-type mice and AT1A receptor–deficient mice by 32.8 ± 2.6% and 60.7 ± 2.7%, respectively, to 10.3 ± 1.2 and 6.0 ± 0.4 μm, respectively. The magnitude of the percent-change in afferent arteriolar diameter was significantly greater than that observed in wild-type mice and prevented by papillectomy. Afferent arteriolar diameter after papillectomy averaged 18.9 ± 0.7 μm.
The critical roles of angiotensin II and AT\textsubscript{1} receptor in determining the magnitude of the TGF response have been suggested by previous studies that demonstrated the absence of the TGF response in ACE-deficient mice\textsuperscript{4} and AT\textsubscript{1A} receptor–deficient mice.\textsuperscript{5} Immunohistochemical studies have demonstrated the presence of AT\textsubscript{1} receptors on macula densa cells\textsuperscript{6} and afferent arteriole smooth muscle cells,\textsuperscript{7} suggesting that angiotensin II may modulate the TGF signal transmission process (1) through modulation of the sensor function by activating AT\textsubscript{1} receptors on the macula densa and (2) through enhancing effector sensitivity by stimulating AT\textsubscript{1} receptors on afferent arterioles. Thus, AT\textsubscript{1A} receptors on both the macula densa and the afferent arteriole may account for nNOS activity contributing to the delayed counteracting modulation of the TGF-mediated afferent arteriole constriction.

Enhanced expression of macula densa nNOS protein and mRNA has been observed in angiotensinogen gene–deficient mice\textsuperscript{8} and AT\textsubscript{1A} receptor–deficient mice,\textsuperscript{9} suggesting that angiotensin II may downregulate nNOS activity at the protein and mRNA levels via an AT\textsubscript{1A} receptor present on the membranes of macula densa cells. Consequently, elevation of NO generation by the increased macula densa nNOS activity in AT\textsubscript{1A} receptor–deficient mice may account for the enhanced counteracting modulation of the TGF-mediated afferent arteriole constriction. However, the role of AT\textsubscript{1} receptors in the regulation of macula densa nNOS expression seems controversial, because renal cortical nNOS protein expression is suppressed in rats after administration of the AT\textsubscript{1} receptor antagonist losartan.\textsuperscript{10}

Acute interaction between angiotensin II and nNOS-derived NO has not been observed in the regulation of afferent arteriolar diameter.\textsuperscript{7} Nevertheless, the present study demonstrated an enhanced constrictor response of afferent arterioles to selective nNOS inhibition in AT\textsubscript{1A} receptor–deficient mice, suggesting that absence of the AT\textsubscript{1A} receptor increases the effect of nNOS on afferent arterioles. Previous studies have demonstrated that angiotensin II stimulates superoxide production via AT\textsubscript{1} receptors in rat aortic rings,\textsuperscript{11} and that superoxide-derived metabolites are expressed in the interstitium and the extraglomerular mesangial cells between the macula densa and the afferent arteriole in the 2-kidney, 1-clip Goldblatt hypertensive rats.\textsuperscript{12} Because superoxide anion inhibits nNOS control of afferent arteriolar diameter by scavenging NO,\textsuperscript{13} the absence of AT\textsubscript{1A} receptor on afferent arteriolar smooth muscle cells can elicit inhibition of superoxide production, leading to an increase in NO bioavailability. Thus, increased NO bioavailability may contribute to the enhanced counteracting modulation of the TGF-mediated biphasic afferent arteriolar constriction. Activation of AT\textsubscript{1} receptors on the afferent arteriole may enhance TGF sensitivity through reducing the bioavailability of NO released from the macula densa by increasing superoxide concentrations between the macula densa and the afferent arteriole. This concept is consistent with a recent study\textsuperscript{14} that demonstrated that pharmacological AT\textsubscript{1} receptor blockade with candesartan diminishes oxidative stress and thus restores the ability of the nNOS inhibitor 7-nitro-indazole to increase proximal stop flow pressure responses.

The increased distal nephron delivery in response to acetazolamide caused significant initial and sustained constrictor responses of afferent arterioles in AT\textsubscript{1A} receptor–deficient mice. In addition, interruption of distal delivery to the macula densa segment by papillectomy caused a significant increase in the afferent arteriolar diameter compared with its basal level. In AT\textsubscript{1A} receptor–deficient mice, however, acetazolamide did not alter the initial response, but the sustained response was significantly attenuated compared with that of wild-type mice, and the attenuation was not observed during selective nNOS inhibition with L-SMTC. These results suggest that AT\textsubscript{1A} receptors contribute to the delayed counteracting vasoconstrictor response that is part of the biphasic TGF-mediated changes in afferent arteriolar diameter that occur during acetazolamide treatment, and that this effect depends on nNOS activity.

Discussion

The main finding in the present study was that the attenuation of the TGF response observed in AT\textsubscript{1A} receptor–deficient mice involves enhanced counteracting modulation by the increased influences of nNOS. In wild-type mice, distal nephron delivery pharmacologically increased by acetazolamide caused a biphasic afferent arteriolar constrictor response consisting of immediate vasoconstriction (initial response) followed by a modest waning (sustained response). Interruption of distal nephron volume and sodium chloride delivery to the macula densa segment by transection of the loops of Henle abolished the afferent arteriolar constrictor responses to acetazolamide and caused a slight increase in the afferent arteriolar diameter compared with its basal level. In AT\textsubscript{1A} receptor–deficient mice, however, acetazolamide did not alter the initial response, but the sustained response was significantly attenuated compared with that of wild-type mice, and the attenuation was not observed during selective nNOS inhibition with L-SMTC. Therefore, the increased distal nephron delivery in response to acetazolamide caused significant initial and sustained constrictor responses of afferent arterioles in AT\textsubscript{1A} receptor–deficient mice. In addition, interruption of distal delivery to the macula densa segment by papillectomy caused a significant increase in the afferent arteriolar diameter of AT\textsubscript{1A} receptor–deficient mice. These results suggest the occurrence of tubular flow–dependent control of afferent arteriolar diameters in AT\textsubscript{1A} receptor–deficient mice. Because angiotensin II activates type 2 receptors do not contribute to the effect of angiotensin II on TGF responses,\textsuperscript{15} AT\textsubscript{1B} receptors may play a role in maintaining the TGF mechanism in the absence of AT\textsubscript{1A} receptors. Alternatively, these data suggest that angiotensin enhances, but does not mediate, the TGF response via AT\textsubscript{1A} receptors, consistent with numerous previous studies.\textsuperscript{1}

The absence of TGF responses in AT\textsubscript{1A} receptor–deficient mice was previously observed by Schnermann et al,\textsuperscript{3} and the discrepancy from the findings in the present study can be explained in several ways. First, there is a difference in gene targeting to generate a strain of AT\textsubscript{1A} germline-null mutant mice.\textsuperscript{21,22} These 2 strains of AT\textsubscript{1A} receptor–deficient mice...
may have differences in the development of the kidney. Second, we assessed the TGF response by measuring decreases in afferent arteriolar diameter instead of by evaluating changes in proximal stop flow pressure. Possible changes in efferent arteriolar tone during stimulation of the TGF mechanism may be responsible for the absence of changes in proximal stop flow pressure. Third, the present study measured the afferent arteriolar diameter of juxtamedullary nephrons, whereas micropuncture approaches to the measurement of proximal stop flow pressure are generally performed in superficial cortical nephrons. The AT1A receptors of juxtamedullary nephrons may make less quantitative contribution to the modulation of the TGF mechanism than those of the superficial cortical nephrons. Distinct from superficial cortical circulation, however, juxtamedullary circulation provides blood flow to the renal medulla, which plays a major role in sodium homeostasis and in regulating the urinary concentrating mechanism, and the TGF response of juxtamedullary nephrons is greater than that of superficial cortical nephrons. Because ACE inhibition elicits a greater increase in glomerular filtration rate of juxtamedullary nephrons compared with superficial cortical nephrons, less contribution of the AT1A receptors to the modulation of the TGF mechanism is unlikely in juxtamedullary nephrons.

Because nNOS influences on afferent arteriolar diameters were greater in AT1A receptor–deficient mice than in wild-type mice, afferent arteriolar diameters of AT1A receptor–deficient mice should be increased compared with wild-type mice. However, the present study demonstrated similar basal diameters of both mice. In addition, the initial response of the TGF-mediated afferent arteriolar constriction was similar in both mice, although angiotensin II is known to enhance the TGF-mediated afferent arteriolar constriction. The kidneys of AT1A receptor–deficient mice may acquire compensatory vasoconstrictor mechanisms that overcome the increased vasodilator influences and contribute to the maintained basal afferent arteriolar diameters and the unchanged initial response of the TGF-mediated afferent arteriolar constriction. However, further studies are needed to resolve afferent arteriolar regulation in AT1A receptor–deficient mice.

Perspectives
Angiotensin II enhances the TGF-mediated afferent arteriolar constriction. The present study demonstrated that AT1A receptors in juxtamedullary afferent arterioles prevent the counteracting resetting process of the biphasic afferent arteriolar constrictor responses to acetazolamide in a manner that is dependent on nNOS activity. Therefore, AT1A receptors may enhance the TGF-mediated afferent arteriolar constriction, at least in part, through reducing the compensatory influences of nNOS on its sustained constrictor responses. These findings suggest that the interactive influences of nNOS and AT1A receptors need to be considered as one part of the complex autoregulatory mechanisms of renal circulation.

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References


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