Nitric Oxide Produced by THAL Nitric Oxide Synthase Inhibits TGF

Hong Wang, Oscar A. Carretero, Jeffrey L. Garvin

Abstract—Nitric oxide (NO) produced by neuronal NO synthase (nNOS) in the macula densa decreases tubuloglomerular feedback (TGF). NO produced by NOS in the thick ascending limb (THAL) inhibits NaCl transport. We hypothesized that NO produced by NOS in the THAL reaches the macula densa and inhibits TGF. Rabbit afferent arterioles and attached macula densa were simultaneously micropерfused in vitro. TGF response was determined by measuring afferent arteriole diameter before and after increasing NaCl in the macula densa perfusate. When the nNOS inhibitor 7-nitroindazole (7-NI) (10 μmol/L) was added to the macula densa lumen, it increased TGF from 2.3±0.2 to 3.5±0.5 μm (P<0.02; n=6). In the presence of 7-NI, Nω-nitro-L-arginine methyl ester (L-NAME) (1 mmol/L) enhanced TGF from 2.6±0.3 to 4.0±0.5 μm (P<0.02; n=6) when the macula densa was perfused orthograde via the THAL, whereas it had no effect on TGF when the macula densa was perfused retrograde via the distal tubule (DT). Inhibition of macula densa soluble guanylate cyclase with LY83583 (1 μmol/L) was added to the macula densa lumen, it increased TGF from 2.3±0.2 to 3.5±0.5 μm (P<0.02; n=6). In the presence of 7-NI, Nω-nitro-L-arginine methyl ester (L-NAME) (1 mmol/L) enhanced TGF from 2.6±0.3 to 4.0±0.5 μm (P<0.02; n=6) when the macula densa was perfused orthograde via the THAL, whereas it had no effect on TGF when the macula densa was perfused retrograde via the distal tubule (DT). Inhibition of macula densa soluble guanylate cyclase with LY83583 (1 μmol/L) blocked the effect of NO produced by THAL NOS when the macula densa was perfused via the THAL. We concluded that NO produced by THAL NOS acts as a paracrine factor, reaching the macula densa and inhibiting TGF. (Hypertension. 2002;39[part 2]:662-666.)

Key Words: nitric oxide | rabbits | arterioles

The macula densa detects changes in NaCl concentration of the luminal fluid at the distal end of the thick ascending limb (THAL). When NaCl concentration increases, the macula densa transmits a signal that constricts the afferent arteriole and decreases glomerular capillary pressure, a process referred to as tubuloglomerular feedback (TGF). TGF is thought to be initiated by cotransport of Na, K, and 2Cl across the luminal membrane of the ascending limb (THAL). When NaCl concentration increases, the macula densa transmits a signal that constricts the afferent arteriole and decreases glomerular capillary pressure, a process referred to as tubuloglomerular feedback (TGF). TGF response was determined by measuring afferent arteriole diameter before and after increasing NaCl in the macula densa perfusate. When the nNOS inhibitor 7-nitroindazole (7-NI) (10 μmol/L) was added to the macula densa lumen, it increased TGF from 2.3±0.2 to 3.5±0.5 μm (P<0.02; n=6). In the presence of 7-NI, Nω-nitro-L-arginine methyl ester (L-NAME) (1 mmol/L) enhanced TGF from 2.6±0.3 to 4.0±0.5 μm (P<0.02; n=6) when the macula densa was perfused orthograde via the THAL, whereas it had no effect on TGF when the macula densa was perfused retrograde via the distal tubule (DT). Inhibition of macula densa soluble guanylate cyclase with LY83583 (1 μmol/L) was added to the macula densa lumen, it increased TGF from 2.3±0.2 to 3.5±0.5 μm (P<0.02; n=6). In the presence of 7-NI, Nω-nitro-L-arginine methyl ester (L-NAME) (1 mmol/L) enhanced TGF from 2.6±0.3 to 4.0±0.5 μm (P<0.02; n=6) when the macula densa was perfused orthograde via the THAL, whereas it had no effect on TGF when the macula densa was perfused retrograde via the distal tubule (DT).

Methods

We used methods similar to those described previously to isolate and microperfuse the afferent arteriole with attached glomerulus and macula densa.12,13 Male New Zealand white rabbits (1.6 to 1.8 kg) were fed standard rabbit chow with 0.34% Na and 0.40% Cl (Ralston Purina) and given tap water ad libitum. They were anesthetized with ketamine plus xylazine (50 mg/kg and 10 mg/kg IM) and sodium pentobarbital (30 mg/kg IV), and heparin (500 U IV) was injected to block coagulation. The kidneys were removed and sliced along the longitudinal corticomedullary axis. Slices were placed in ice-cold minimum essential medium (MEM; Gibco) containing 5% BSA (Sigma) and dissected under a stereomicroscope (SZH; Olympus). From each rabbit, a single superficial afferent arteriole and its intact glomerulus were microdissected together with adherent tubular segments consisting of portions of the THAL, macula densa, and early distal tubule (DT). Using a micropipette, the sample was transferred to a temperature-regulated chamber mounted on an inverted microscope (IMT-2; Olympus) with Hoffmann modulation. Both the afferent arteriole and the end of the DT (or THAL) were cannulated with an array of glass pipettes.12,13 Intraluminal pressure of the afferent arteriole was measured by Landis' technique, using a fine pipette introduced into the lumen through the perfusion pipette. The afferent arteriole was perfused with MEM containing 5% BSA, and intraluminal pressure was maintained at 60 mm Hg throughout the experiment.

The bath was MEM containing 0.15% BSA and was exchanged continuously at a rate of 1 mL/min. Microdissection and cannulation were completed at 8°C, after which the bath was gradually warmed to 37°C. Once temperature was stable, a 30-minute equilibration period was allowed before any measurements were taken.
Microperfusion of the end of the DT or THAL was started with low-NaCl solution (5 mmol/L Na⁺; 3 mmol/L Cl⁻) containing the following composition (in mmol/L): 10 HEPES, 3 KCl, 1.2 MgSO₄, 2 K₂PO₄, 5 NaHCO₃, 5.5 glucose and 1 calcium lactate (pH 7.4). The high-NaCl solution had the same composition except that 74 mmol/L NaCl was added; thus the final concentration was 79 mmol/L Na⁺ and 77 mmol/L Cl⁻.

Images were displayed at magnifications up to ×1980 and recorded with a Sony video system consisting of a camera (DXC-755), monitor (PVM1342Q), and video recorder (EDV-7500). We defined TGF as the change in afferent arteriole diameter when the NaCl concentration perfusing the macula densa was increased from low to high. Diameter was measured with an image analysis system (Universal Imaging).

7-nitroindazole (7-NI) (Cayman), an inhibitor of nNOS, was dissolved in 98% alcohol by sonication. The final alcohol concentration was 0.018%, which in preliminary experiments did not affect TGF response. This compound has been reported to have a half-maximal inhibitory concentration (IC₅₀) of 0.47 µmol/L and has been shown to effectively and selectively block synthesis of NO by nNOS.¹⁴ N-nitro-L-arginine methyl ester (L-NAME), a nonselective NOS inhibitor, was purchased from Sigma; LY83583, a soluble guanylate cyclase inhibitor, was obtained from Biomol Laboratories.

**Statistics**

Data are expressed as mean±SEM. Data were analyzed using ANOVA for repeated measures. Post-hoc testing was performed using paired t tests. Hochberg’s method was used to adjust for multiple testing. *P*<0.05 was considered significant.

**Results**

To show that NO produced by macula densa nNOS blunts TGF, we first perfused the macula densa retrograde via the DT with 7-NI (10 µmol/L). During the control period, TGF was 2.3±0.2 µm, because afferent arteriole diameter decreased from 15.8±0.5 to 13.5±0.5 µm. After 7-NI was added to the macula densa perfusate, diameter decreased by 3.5±0.5 µm, from 15.6±0.5 to 12.1±0.5 µm (*P*<0.02 versus control TGF; *n*=6) (Figure 1).

To examine whether NO produced by the THAL alters TGF, we perfused the macula densa orthograde via the THAL. First, we perfused the macula densa with 7-NI to inhibit macula densa nNOS and measured TGF. In the presence of 7-NI, afferent arteriole diameter decreased by 2.6±0.3 µm (from 17.2±0.5 to 14.6±0.5 µm) when macula densa NaCl was increased. Then we added L-NAME to the perfusate to inhibit THAL NOS. After L-NAME (1 mmol/L) was added to the macula densa lumen in the presence of 7-NI, diameter decreased by 4.0±0.5 µm, from 17.2±0.7 to 13.2±1.0 µm (*P*<0.02 versus 7-NI alone; *n*=6) (Figure 2).

To show that the effect of L-NAME was due to inhibition of NOS in the THAL rather than nNOS in the macula densa or some nonspecific effect, we perfused the macula densa retrograde via the DT. First, we perfused the macula densa with 7-NI to inhibit macula densa NOS and measured TGF. With 7-NI in the macula densa lumen, afferent arteriole diameter decreased by 2.9±0.2 µm (from 16.0±0.6 to 13.1±0.5 µm) when macula densa NaCl was increased. When we added L-NAME to the macula densa lumen in the presence of 7-NI, diameter decreased by 2.8±0.5 µm, from 15.8±0.6 to 13.0±0.5 µm (*P*<0.9 versus 7-NI alone; *n*=6) (Figure 3).

Finally, we examined whether inhibiting soluble guanylate cyclase in the macula densa blocks the effect of THAL-derived NO. When the macula densa was perfused orthograde via the THAL with 1 µmol/L LY83583, afferent arteriole diameter decreased by 3.5±0.6 µm (from 16.7±0.9 to 13.2±1.1 µm) when macula densa NaCl was increased. After L-NAME (1 mmol/L) was added to the macula densa lumen in the presence of LY83583, diameter decreased by 3.4±0.6 µm, from 16.3±0.8 to 12.9±1.1 µm (*P*<0.2 versus LY83583 alone; *n*=5) (Figure 4).

**Discussion**

Many investigators have shown that NO derived from the macula densa inhibits TGF.¹⁵–¹⁷ Here we have confirmed this using 7-NI, which inhibits nNOS. In addition, we found that if we first inhibited nNOS with 7-NI and then inhibited eNOS and iNOS with L-NAME, a nonselective NOS inhibitor, we potentiated TGF when the macula densa was perfused via the THAL but not when it was perfused via the DT. These data suggest that NO produced by either eNOS or iNOS in the THAL inhibits TGF.

Previously we have shown that inhibiting soluble guanylate cyclase in the macula densa blocks the effect of macula
We examined whether inhibiting soluble guanylate cyclase in the macula densa blocks the effect of THAL-derived NO. When the macula densa was perfused orthograde via the THAL with LY83583, a soluble guanylate cyclase inhibitor, L-NAME had no effect on TGF, suggesting that inhibition of soluble guanylate cyclase in the macula densa blocks the effect of NO produced by THAL NOS. These experiments also suggest that NO derived from the THAL acts at the macula densa level and not by diffusing to the afferent arterioles.

NO generated in the tubule or peritubular capillaries has the potential to influence tubular transport, thereby altering the incoming TGF signal at the macula densa. Inhibiting the effect of NO on THAL transport would tend to enhance TGF. We have shown that the THAL produces NO, which acts as an autacoid and inhibits transport. However, it is unlikely that the effect of L-NAME on the THAL (and thus the NaCl concentration at the macula densa) can account for our results. The flow rate we used to perfuse the macula densa is great enough that THAL NaCl absorption cannot change the NaCl concentration of the perfusate significantly.

Figure 2. Effect of perfusing the macula densa with L-NAME orthograde via the THAL on TGF in the presence of 7-NI. Left panel shows afferent arteriole diameter (n=6; ***P<0.001 vs low NaCl plus 7-NI; ###P<0.001 vs low NaCl plus 7-NI and L-NAME). Right panel shows TGF response (*P<0.02 vs 7-NI alone).

Figure 3. Effect of perfusing the macula densa with L-NAME retrograde via the DT on TGF in the presence of 7-NI. Left panel shows afferent arteriole diameter (n=6; ***P<0.001 vs low NaCl plus 7-NI; ##P<0.01 vs low NaCl plus 7-NI and L-NAME). Right panel shows TGF response. There was no significant difference in TGF with 7-NI alone and 7-NI plus L-NAME.
L-NAME significantly enhanced TGF by perfusing the macula densa orthograde via the THAL, whereas it had no effect on TGF when the macula densa was perfused retrograde via the DT. These results provide direct evidence that the THAL may indeed produce NO, which in turn modulates TGF. Inhibition of soluble guanylate cyclase in the macula densa blocks the effect of NO produced by THAL NOS. We conclude that NO produced by THAL NOS acts as a paracrine factor, reaching the macula densa and inhibiting TGF.

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References


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