Inhibition of Apical Na\(^+\)/H\(^+\) Exchangers on the Macula Densa Cells Augments Tubuloglomerular Feedback

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

**Abstract**—NO produced by neuronal NO synthase (nNOS) in the macula densa blunts tubuloglomerular feedback (TGF). nNOS activity is strongly pH-dependent. Increasing luminal NaCl concentration increases nNOS activity, NO production, and apical Na\(^+\)/H\(^+\) exchange. Na\(^+\)/H\(^+\) exchange alkalinizes the macula densa. We hypothesized that inhibiting apical Na\(^+\)/H\(^+\) exchange in macula densa cells would augment TGF by blunting nNOS activation caused by increasing luminal NaCl concentration. Rabbit afferent arterioles and attached macula densas were micropерfused in vitro. TGF response was defined as the change in afferent arteriole diameter caused by increasing the NaCl concentration in the macula densa perfusate. 7-Nitroindazole (7-NI; 10 μmol/L) alone in the macula densa lumen increased the TGF response from 2.4±0.1 to 3.8±0.2 μm (P<0.01). When dimethyl amiloride (100 μmol/L), a Na\(^+\)/H\(^+\) exchange inhibitor, was added to the macula densa lumen, it increased the TGF response from 2.5±0.3 to 3.7±0.5 μm (P<0.01). In the presence of dimethyl amiloride, 7-NI had no effect on the TGF response (from 2.6±0.2 to 2.7±0.2 μm). Our data indicate that inhibiting apical Na\(^+\)/H\(^+\) exchange in the macula densa mimics the effect of inhibiting NO production by nNOS in the macula densa on TGF. Thus, it is possible that increased apical Na\(^+\)/H\(^+\) exchange caused by increasing the sodium concentration in the lumen of the macula densa activates macula densa nNOS. The link between nNOS and Na\(^+\)/H\(^+\) exchange may be intracellular pH. (Hypertension. 2003;41[part 2]:688-691.)

**Key Words**: nitric oxide ■ rabbit ■ tubuloglomerular feedback ■ afferent arteriole ■ loop of Henle

The macula densa is a unique plaque of 10 to 15 cells located within the cortical thick ascending limb and in close association with its own glomerulus. Macula densa cells express Na\(^+\)/H\(^+\) exchangers at the apical and basolateral membranes. The apical Na\(^+\)/H\(^+\) exchange likely participates in Na\(^+\) transport and thus may play a role in tubuloglomerular feedback (TGF).\(^1\)–\(^5\) Increasing the luminal Na\(^+\) concentration at the macula densa augments apical Na\(^+\)/H\(^+\) exchange. This in turn leads to increased Na\(^+\) entry, H\(^+\) extrusion, and alkalinization of macula densa cells.\(^1\)–\(^5\)

NO produced by neuronal NO synthase (nNOS) in the macula densa blunts TGF. Increasing luminal NaCl concentration increases nNOS activity and NO production, as evidenced by inhibitor studies\(^6\)–\(^11\) and direct measurements of macula densa NO.\(^12\) Presently, the mechanism by which nNOS in the macula densa is activated is unclear. One possible mechanism is via changes in intracellular pH (pH). NOS activity is strongly pH-dependent, reaching a maximum at a pH of ≈7.5 to 8.0.\(^13\)\(^14\) Physiological changes in pH can have profound effects on nNOS activity and NO production, even in the presence of saturating concentrations of Ca\(^2+\) and its cofactors.\(^14\) Such increases in nNOS activity may be owing to the rise in pH caused by increasing luminal NaCl and activation of Na\(^+\)/H\(^+\) exchange. Consequently, we hypothesized that inhibiting apical Na\(^+\)/H\(^+\) exchange in macula densa cells blunts the activation of nNOS caused by a change in luminal NaCl concentration and augments TGF by reducing NO production.

**Methods**

We used methods similar to those described previously to isolate and micropерfuse the afferent arteriole with attached glomerulus and macula densa.\(^15\)\(^16\) Male New Zealand white rabbits (Covance Research Products, Kalamazoo, Mich; 1.3 to 2.2 kg) were fed standard rabbit chow with 0.34% NaCl 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 (Gibco) containing 5% BSA (Sigma) and dissected under a stereoscope (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 thick ascending limb, macula densa, and early distal tubule. By using a micropipette, the preparation 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 distal tubule were cannulated with an array of glass pipettes.\(^15\)\(^16\) Intraluminal pressure of the afferent arteriole was measured by Landis’ technique by using a fine pipette introduced into the lumen through the perfusion

Received October 9, 2002; first decision October 28, 2002; revision accepted November 14, 2002.
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**Hypertension** is available at http://www.hypertensionaha.org

DOI: 10.1161/01.HYP.0000048863.75711.B2

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pipette. The afferent arteriole was perfused with minimum essential medium containing 5% BSA, and intraluminal pressure was maintained at 60 mm Hg throughout the experiment.

The bath was minimum essential medium containing 0.15% BSA and was exchanged continuously at a rate of 1 mL/min. Micropuncture 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.

The macula densa was micropерфused with low-NaCl solution (10 mmol/L Na+, 9 mmol/L Cl− containing (mmol/L): 10 HEPES, 1 CaCO₃, 0.5 K₂HPO₄, 4 KHCO₃, 1.2 MgSO₄, 5.5 glucose, 0.5 sodium acetate, and 0.5 sodium lactate (pH 7.4). The high-NaCl solution had the same composition except that 79 mmol/L NaCl was added; thus, the final concentration was 80 mmol/L Na+ and 79 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).

Dimethyl amiloride (Sigma) was prepared daily as a 10 mmol/L stock in warm perfusion solution and diluted to 100 μmol/L just before the experiment. 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. 7-NI has been shown to be a selective inhibitor of macula densa nNOS both in experiments using isolated perfused juxtaglomerular apparatus9,10,17 and the in vivo micropuncture preparation.5–8,11

### Results

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

#### Data

First, we examined the effect of nNOS inhibition on TGF. To show that NO produced by nNOS blunts TGF, we added 7-NI (10 μmol/L) to the macula densa perfusate to block NO production and measured TGF. During the control period, afferent arteriole diameter decreased from 16.0±0.4 to 16.2±0.4 μm when the luminal NaCl concentration was increased. After 7-NI was added to the macula densa perfusate, diameter decreased from 16.0±0.4 to 12.2±0.4 μm. Thus, the TGF response increased from 2.4±0.1 to 3.8±0.2 μm after nNOS was inhibited by 7-NI (P<0.01; n=6). Control experiments showed no significant change in TGF response with time (Figure 1).

To investigate whether blocking apical Na+/H+ exchange augments TGF, we compared TGF responses before and after inhibiting apical Na+/H+ exchange with dimethyl amiloride (100 μmol/L). During the control period, afferent arteriole diameter decreased from 15.7±0.3 to 13.2±0.4 μm when the luminal NaCl concentration was increased. After dimethyl amiloride was added to the macula densa perfusate, diameter decreased from 15.5±0.4 to 11.8±0.6 μm. Thus, blocking apical Na+/H+ exchange increased the TGF response from 2.5±0.3 to 3.7±0.5 μm (P<0.01; n=6) Control experiments showed no significant change in TGF response with time (Figure 2).

Next, we investigated whether the increase in TGF caused by inhibiting NO production by nNOS requires a functional apical Na+/H+ exchanger. For this, we tested the effect of the nNOS inhibitor 7-NI on TGF with dimethyl amiloride present in the macula densa perfusate. In the presence of 100 μmol/L dimethyl amiloride, afferent arteriole diameter decreased from 16.7±0.7 to 14.1±0.8 μm when the luminal NaCl concentration was increased. After we added 7-NI (10 μmol/L) to the macula densa lumen with dimethyl amiloride present, diameter decreased from 16.6±0.8 to 13.9±0.8 μm when the luminal NaCl concentration was increased. Thus, TGF response was unchanged by nNOS inhibition after blocking apical Na+/H+ exchange with dimethyl amiloride (2.6±0.2 versus 2.7±0.2 μm; P>0.4; n=6) (Figure 3).

#### Discussion

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Figure 1. Effect of perfusing the macula densa with 7-NI (10 μmol/L) on TGF. Left, Afferent arteriole diameter (n=6; **P<0.001 vs low NaCl; ††P<0.001 vs low NaCl plus 7-NI). Right, TGF response (P<0.01 vs control TGF).

Figure 2. Effect of perfusing the macula densa with dimethyl amiloride (DMA; 100 μmol/L) on TGF. Left, Afferent arteriole diameter (n=6; **P<0.001 vs low NaCl; ††P<0.001 vs low NaCl plus dimethyl amiloride). Right, TGF response (P<0.01 vs control TGF).
whether functional apical and/or basolateral Na⁺/H⁺ exchangers are required for activation. To do so, we studied the ability of the Na⁺/H⁺ exchanger inhibitor dimethyl amiloride to blunt the effect of nNOS inhibition on TGF when added to the macula densa perfusate or bath. We found that blocking nNOS with 7-NI stimulated TGF as reported previously. Inhibiting apical Na⁺/H⁺ exchange by adding dimethyl amiloride alone to the macula densa perfusate also enhanced TGF. Because inhibiting either nNOS or Na⁺/H⁺ exchange augmented TGF, we examined whether the stimulation of TGF caused by nNOS inhibition could be prevented by blocking apical Na⁺/H⁺ exchange. When we inhibited macula densa nNOS with 7-NI in the presence of dimethyl amiloride, 7-NI had no effect on TGF. Thus, inhibition of Na⁺/H⁺ exchange prevented the effects of nNOS blockade.

There are several possible explanations for our data. First, apical Na⁺/H⁺ exchangers may be directly linked to nNOS via protein/protein interactions. Although not reported for nNOS, both inducible and endothelial NOS are regulated through interactions involving a number of regulatory proteins. Second, nNOS activation may be linked to sodium influx. However, this seems unlikely because the Na⁺/H⁺ exchanger only transports a small fraction of the sodium that enters the cell when luminal NaCl concentration is elevated, whereas most sodium enters via the Na/K/2Cl cotransporters. Changes in intracellular calcium are also a possibility, because nNOS is activated by an increase in intracellular calcium. However, both increases and decreases in intracellular calcium in the macula densa have been reported when the luminal NaCl concentration rises. The last possibility is a change in pH.

Perfusion of the macula densa lumen with solutions containing high concentrations of sodium leads to an increase in NO production and rapid alkalinization of macula densa cytoplasm. For instance, when luminal sodium is increased, pH rises from 7.2 to between 7.4 and 7.8 depending on the increase in NaCl. This increase in pH has been attributed to activation of apical amiloride-sensitive Na⁺/H⁺ exchange. Production of NO by nNOS is strongly pH-dependent. The enzyme has maximal activity at a pH of 8. NOS activity increases 5-fold with alkalinization across a physiologically relevant range. Therefore, our data may be explained as follows. Increasing the NaCl concentration at the macula densa stimulates Na⁺/H⁺ exchange owing to a large inward-directed sodium gradient. Activation of the exchanger results in alkalinization of the macula densa. The increase in pH enhances nNOS activity and NO production. The increased NO levels blunt TGF.

In vitro studies have shown that inhibiting nNOS only affects afferent arteriole diameter when the NaCl concentration in the macula densa is high. These data indicate that nNOS is inactive when luminal sodium is low and is activated when the sodium concentration increases. In vivo micropuncture experiments show analogous results. Furthermore, direct measurements of NO have shown that NO production increases with an increase in NaCl in the lumen of the macula densa. Our data offer a possible explanation for such findings. With low sodium concentrations in the macula densa lumen, nNOS cannot affect TGF because there is little or no NO production owing to the relatively acidic pH of the macula densa.

**Perspectives**

In summary, blocking Na⁺/H⁺ exchange in the macula densa significantly enhanced TGF. By itself the nNOS inhibitor 7-NI in the macula densa lumen augmented TGF, but in the presence of a Na⁺/H⁺ exchange inhibitor, dimethyl amiloride, 7-NI had no effect on TGF. These data indicate that inhibiting apical Na⁺/H⁺ exchange in the macula densa mimics the effect of inhibiting NO production by nNOS in the macula densa on TGF. Thus, it is possible that increased apical Na⁺/H⁺ exchange caused by increasing the sodium concentration in the lumen of the macula densa activates macula densa nNOS. The link between nNOS and Na⁺/H⁺ exchange may be an increase in pH caused by the latter; however, we did not measure changes in macula densa pH in this study. This may provide a feedback mechanism that prevents extreme TGF responses. Our data may also explain why macula densa nNOS inhibition has no effect on afferent arteriole diameter when the NaCl concentration in the macula densa lumen is low.

**Acknowledgments**

This work was partly supported by grants from the National Heart, Lung and Blood Institute (HL-28982 to O.A.C. and HL-70985 to J.L.G.).

**References**


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Hypertension. 2003;41:688-691; originally published online December 30, 2002;
doi: 10.1161/01.HYP.0000048863.75711.B2
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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