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 microperfused 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⁺ exchanger likely participates in Na⁺ transport and thus may play a role in tubuloglomerular feedback (TGF). Increasing luminal Na⁺/H⁺ exchange increases NO production and NO exchange in the macula densa. Presently, the mechanism by which nNOS activity is strongly pH-dependent, reaching a maximum at a pH of ≈7.5 to 8.0. Physiological changes in pH can have profound effects on nNOS activity and NO production, in the presence of saturating concentrations of Ca²⁺ and its cofactors. 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 microperfuse the afferent arteriole with attached glomerulus and macula densa. Male New Zealand white rabbits (Covance Research Products, Kalamazoo, Mich; 1.3 to 2.2 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 (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 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. 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.

From the Hypertension and Vascular Research Division, Department of Internal Medicine, Henry Ford Hospital (H.W., O.A.C., J.L.G.), Detroit, Mich; and Department of Cardiology, Zhongnan Hospital of Wuhan University (H.W.), Wuhan, People’s Republic of China.

Correspondence to Jeffrey L. Garvin, PhD, Hypertension and Vascular Research Division, Department of Internal Medicine, Henry Ford Hospital, 2799 W Grand Blvd, Detroit, MI 48202. E-mail jgarvin1@hfhs.org

Hypertension is available at http://www.hypertensionaha.org DOI: 10.1161/01.HYP.0000048863.75711.B2

© 2002 American Heart Association, Inc.

688
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. 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.

The macula densa was microperfused with low-NaCl solution (10 mmol/L Na+; 9 mmol/L Cl–) containing (in mmol/L): 10 HEPES, 1 CaCl2, 0.5 KHP04, 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).

The afferent arteriole (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-Nitroindazole (7-NI; Cayman) is known to be a selective inhibitor of macula densa nNOS both in experiments using isolated perfused juxtaglomerular apparatus9,10,17 and in vivo micropuncture preparation.5–8,11

Statistics

Data are expressed as mean ± SEM. 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.

Results

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 ± 0.5 to 13.6 ± 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

We10 and others6,18–21 have shown that NO derived from the macula densa inhibits TGF. However, inhibitor studies6,19–21 and direct measurements of NO in the macula densa12 show that NO production is low or nonexistent when the macula densa lumen contains a low concentration of NaCl and increases when NaCl increases. The mechanism by which the increase in NaCl activates NO production by nNOS is unknown. There are several possible mechanisms by which nNOS may be stimulated in the macula densa. We examined
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. 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 lumen 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**


Inhibition of Apical Na⁺/H⁺ Exchangers on the Macula Densa Cells Augments
Tubuloglomerular Feedback
Hong Wang, Oscar A. Carretero and Jeffrey L. Garvin

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
Copyright © 2002 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://hyper.ahajournals.org/content/41/3/688

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published
in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial
Office. Once the online version of the published article for which permission is being requested is located,
click Request Permissions in the middle column of the Web page under Services. Further information about
this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/