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) 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 macula densa and comprises an intrarenal regulatory system that operates at the single-nephron level to stabilize nephron function.1–3

Nitric oxide (NO) synthesized by neuronal NO synthase (nNOS) in the macula densa is an important modulator of TGF. Micropuncture studies in vivo have shown that infusion of an NO inhibitor into the late proximal tubule can greatly increase TGF response.4 We have reported that the effects of NO produced by the macula densa on TGF are mediated by soluble guanylate cyclase within the macula densa.5

The macula densa is located immediately downstream from the THAL, and several studies have shown that the THAL expresses endothelial NOS (eNOS), inducible NOS (iNOS) and nNOS.6–8 We have demonstrated that the THAL produces NO, which acts as an autacoid and inhibits transport.9–11 Given the proximity of the THAL to the macula densa, we hypothesized that NO produced by THAL NOS reaches the macula densa and inhibits TGF.

Methods

We used methods similar to those described previously to isolate and micropерfuse 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 K₂HPO₄, 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 recorded with a Sony video system consisting of a camera (DXC-1980 and PVM1342Q), monitor (PVM1342Q), and video recorder (EDV-7500). A 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 0.01%, 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. A N⁵-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 orthorad 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 NO 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 nNOS 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<0.02 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 orthorad 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. 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. Furthermore, because inhibition of soluble guanylate cyclase in the macula densa blocked the TGF potentiation caused by L-NAME, this suggests that it was not due to a further increase in NaCl in the macula densa.

Several studies have shown that the THAL expresses eNOS, iNOS, and nNOS. Although we did not address this issue directly, we can eliminate nNOS. After we used a nNOS inhibitor (7-NI) to inhibit nNOS in the macula densa and THAL, a nonselective NOS inhibitor (L-NAME) significantly enhanced TGF when the macula densa was perfused via the THAL but not via the DT, suggesting that NO produced by eNOS and iNOS in the THAL inhibits TGF. Since we have previously shown that NO produced by THAL eNOS inhibits THAL chloride flux ($J_{CL}$), the NO that inhibits TGF is likely to be produced by eNOS.

NO could either diffuse from the THAL to the macula densa or be carried there by the luminal fluid. NO is a highly diffusible gas that moves freely through tissues. When removed from tissue solely by auto-oxidation to biologically inert nitrite/nitrate, the diffusion distance of NO is calculated to be 100 to 200 μm. At least some THAL cells are adjacent...
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.

Acknowledgment
This work was supported in part by the National Heart, Lung and Blood Institute (HL-28982).

References

to the macula densa, so that NO could diffuse from these cells to the macula densa. However, in our preparation the THAL is about 500 μm long. NO produced by the THAL may be carried to the macula densa by the luminal perfusate rather than via diffusion. NO exiting the THAL via the basolateral membrane is unlikely to play a role in our experiments, because the bath is rapidly exchanged and would carry NO away from the macula densa rather than toward it. However, in vivo this is not the case.

Given that macula densa nNOS produces large quantities of NO, one might well ask, what is the physiological consequence of NO from the THAL reaching the macula densa and inhibiting TGF? Under various physiological circumstances, the effect of THAL-derived NO may become more or less important. For instance, when animals are placed on a high salt diet, the expression of nNOS in the macula densa dramatically declines, as does the amount of NO produced. Consequently, the regulation of TGF by macula densa-derived NO is greatly diminished. However, under this same circumstance eNOS expression in THALs is greatly enhanced, as is NO production. Given this, we would predict that NO from the THAL becomes a more important regulator of TGF when animals are placed on a high salt diet.

In addition to differential regulation of NOS expression in the macula densa and THAL, there may also be differential regulation of activity. We have demonstrated that eNOS activity in the THAL is regulated by endothelin and alpha 2 adrenergics, but not through changes in intracellular Ca, the putative regulator of nNOS in the macula densa. Thus, the activity of the various NOS isoforms may be differentially regulated in different hormonal milieus.

Furthermore, the data presented in this manuscript are the first to show that NO produced by a tubular segment can have a paracrine effect on another renal structure. Thus, the importance of the data are not limited in scope to regulation of TGF, but rather TGF can be considered a “bioassay” for the paracrine effects of NO produced by the THAL.

In summary, an nNOS inhibitor (7-NI) in the macula densa dramatically declines, as does the amount of NO produced in the thick ascending limb (THAL) by endogenous nitric oxide synthase (NO) increases during salt loading. J Am Soc Nephrol. 1996;7:1291. Abstract.


Nitric Oxide Produced by THAL Nitric Oxide Synthase Inhibits TGF
Hong Wang, Oscar A. Carretero and Jeffrey L. Garvin

Hypertension. 2002;39:662-666
doi: 10.1161/hy0202.103470

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/39/2/662

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/