Kidney International, Vol. 60 (2001), pp. 1676–1683

Role of neuronal nitric oxide synthase in the macula densa

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Role of neuronal nitric oxide synthase in the macula densa.

Background. There is evidence that macula densa nitric oxide (NO) inhibits tubuloglomerular feedback (TGF). However, TGF response is not altered in mice deficient in neuronal nitric oxide synthase (nNOS) (−/−). Furthermore, nNOS expression in the macula densa is inversely related to salt intake, yet micropuncture studies have shown that NOS inhibition potentiates TGF in rats on high sodium intake but not in rats on a low-salt diet. These inconsistencies may be due to confounding systemic factors, such as changes in circulating renin. To further clarify the role of macula densa nNOS in TGF response, independent of systemic factors, we tested the hypothesis that (*1*) TGF response is inversely related to sodium intake, and (*2*) during low sodium intake, NO produced by macula densa nNOS tonically controls the basal diameter of the afferent arteriole (Af-Art).

Methods. Af-Arts and attached macula densas were simultaneously microperfused in vitro. TGF response was determined by measuring Af-Art diameter before and after increasing NaCl in the macula densa perfusate. TGF response was studied in wild-type (+/+) and nNOS knockout mice (−/−), as well as in juxtaglomerular apparatuses (JGAs) from rabbits fed a low-, normal-, or high-NaCl diet.

Results. TGF responses were similar in nNOS +/+ and −/− mice. However, in nNOS +/+ mice, 7-nitroindazole (7-NI) perfused into the macula densa significantly potentiated the TGF response ($P = 0.001$), while in nNOS −/− mice, this potentiation was absent. In rabbits on three different sodium diets, TGF responses were similar and were potentiated equally by 7-NI. However, in JGAs from rabbits on a low-NaCl diet, adding 7-NI to the macula densa while perfusing it with low-NaCl fluid caused Af-Art vasoconstriction, decreasing the diameter by 14% (from 21.7 ± 1.3 to 18.6 ± 1.5 μm; $P < 0.001$). This effect was not observed in JGAs from rabbits fed a normal- (19.0 ± 0.5 vs. 19.3 ± 0.8 μm after 7-NI) or high-NaCl diet (18.6 ± 0.7 vs. 18.4 ± 0.7 μm).

Conclusions. First, in this in vitro preparation, chronic changes in macula densa nNOS do not play a major role in the regulation of TGF. Compensatory mechanisms may develop during chronic alteration of nNOS that keep TGF relatively constant. Second, nNOS regulates TGF response acutely. Third, the results obtained in the +/+ and −/− mice also confirm that the effect of 7-NI is due to inhibition of macula densa nNOS. Finally, during low sodium intake (without induction of TGF), the regulation of basal Af-Art resistance by macula densa nNOS suggests that NO in the macula densa helps maintain renal blood flow during the high renin secretion caused by low sodium intake.

Key words: microperfusion, tubuloglomerular feedback, glomerular hemodynamics, low sodium diet, 7-nitroindazole.

Received for publication January 4, 2001
and in revised form May 3, 2001
Accepted for publication June 4, 2001

Nitric oxide (NO) plays an important role in the control of glomerular hemodynamics. Various in vivo and in vitro studies have shown that endothelial NO directly regulates afferent and efferent arteriole (Af-Art and Ef-Art) resistance. NO also modulates the vasoconstrictor effect of angiotensin II and myogenic responses to increased perfusion pressure [1-3], while NO produced by neuronal NO synthase (nNOS) in the macula densa decreases the tubuloglomerular feedback (TGF) response [4-8]. In the nephron, nNOS is highly expressed in the cells of the macula densa [9], and studies have shown that NO produced within the macula densa may be important in the regulation of TGF and renin release [7, 10]. We have studied the role of macula densa NO in isolated juxtaglomerular apparatuses (JGA) from rabbits on a normal-sodium diet and found that adding N^{ω}-nitro-L-arginine methyl ester (L-NAME) to the macula densa perfusate increased Af-Art constriction when the macula densa was perfused with high NaCl [11]. We concluded that NO produced within the cells of the macula densa decreases Af-Art constriction when TGF is activated. On the other hand, Schnermann et al, using an in vivo stop-flow technique, reported that TGF response was no different between wild-type (+/+) and nNOS −/− mice and speculated that this may be due to the expression of shorter isoforms of nNOS in the knockout mice [12]. Expression of nNOS in the macula densa is inversely related to salt intake (abstract; Singh et al, *FASEB J* 9:4888, 1995) [13]. However, micropuncture studies have shown that blocking nNOS enhanced TGF response in rats on a high-salt diet, but not in rats on a low-salt diet [14]. Thus, the data concerning NOS expression and the functional studies appear to be contradictory.

To clarify the role of macula densa nNOS in TGF response, we used an in vitro isolated perfused JGA preparation, which is devoid of confounding systemic factors

such as circulating renin or sympathetic nerve activity. We studied whether (*1*) TGF is increased in JGAs from nNOS −/− mice, and whether the potentiation induced by inhibiting macula densa nNOS with 7-nitroindazole (7-NI) is present or absent in these mice; (*2*) chronically feeding rabbits a low-, normal- or high-NaCl diet alters both TGF response and the potentiation induced by inhibition of macula densa nNOS; and (*3*) in JGAs from rabbits fed a low-NaCl diet, inhibition of macula densa nNOS during perfusion of the macula densa with low sodium increases basal Af-Art resistance. This suggests that during low NaCl intake, macula densa nNOS helps maintain renal blood flow, perhaps by antagonizing the vasoconstrictor effect of the renin-angiotensin system.

METHODS

A method similar to those described previously was used to isolate and microperfuse Af-Arts with macula densa attached [15, 16]. Mice and rabbits were anesthetized with ketamine. The kidneys were sliced along the corticomedullary axis; slices were placed in ice-cold minimal essential medium (MEM; Gibco, Grand Island, NY, USA) containing 5% bovine serum albumin (BSA; Sigma Chemical Company, St. Louis, MO, USA) and dissected under a stereomicroscope (Olympus SZH, Olympus Corp., Tokyo, Japan). A single superficial Af-Art and its intact glomerulus from each animal were dissected together with adherent tubular segments consisting of portions of the thick ascending limb of Henle, macula densa, and early distal tubule. Using a micropipette, the sample was transferred to a temperature-regulated chamber mounted on an inverted microscope (Olympus IMT-2, Olympus Corp.) with Hoffmann modulation. Both the Af-Art and the end of either the distal tubule or thick ascending limb were cannulated with an array of glass pipettes.

Intraluminal pressure of the Af-Art was measured by Landis' technique, using a fine pipette introduced into the lumen through the perfusion pipette. The Af-Art was perfused with oxygenated MEM (95% O_2/5% CO_2) containing 5% BSA, and intraluminal pressure was maintained at 60 mm Hg throughout the experiment. The macula densa was perfused with a modified Krebs-Ringer bicarbonate buffer containing either low or high NaCl. The basic composition of the low-NaCl buffer used in the mice was 15 mmol/L $NaHCO_3$, 0.96 mmol/L NaH_2PO_4, 0.24 mmol/L Na_2HPO_4, 5 mmol/L $KHCO_3$, 1.2 mmol/L $MgSO_4$, 1 mmol/L $CaCl_2$, 5.5 mmol/L glucose, and 1 mmol/L Na acetate (Na^+, 17 mEq/L; Cl^-, 2 mEq/L); the high-NaCl buffer was identical except that 48 mmol/L NaCl was added (Na^+, 65 mEq/L; Cl^-, 50 mEq/L). The basic composition of the low-NaCl buffer used in the rabbits was 25 mmol/L $NaHCO_3$, 0.96 mmol/L NaH_2PO_4, 0.24 mmol/L Na_2HPO_4, 5 mmol/L KCl, 1.2 mmol/L $MgSO_4$, 1 mmol/L $CaCl_2$, and 5.5 mmol/L glucose (Na^+, 26 mEq/L; Cl^-, 7 mEq/L). The high-NaCl buffer was identical except that 58 mmol/L NaCl was added (Na^+, 84 mEq/L; Cl^-, 65 mEq/L). Both buffers were oxygenated to pH 7.4. The bath consisted of 100 μL MEM containing 0.15% BSA and was exchanged continuously at a rate of 1 mL/min. Microdissection and cannulation were completed within 90 minutes at 8°C, after which the bath was gradually warmed to 37°C for the rest of the experiment. Once the temperature was stable, a 30-minute equilibration period was allowed before taking any measurements. Images were displayed at magnifications up to 1980× and were recorded with a Sony video system consisting of a camera (DXC-755), monitor (PVM1942), and video recorder (EDV-9500). The diameter of the distal Af-Art was measured with an image analysis system (Universal Imaging, West Chester, PA, USA) at the site of maximal response.

7-nitroindazole (7-NI; Cayman, Ann Arbor, MI, USA), 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 (IC_{50}) of 0.47 μmol/L and has been shown to effectively and selectively block synthesis of NO by nNOS [17].

Experimental protocols

Effect of 7-NI on TGF in nNOS +/+ and −/− mice. Wild-type controls (C57BL/6Jx129/SV) and nNOS −/− mice on a C57BL/6Jx129/SV background (5 to 6 weeks old) were used. After a 20-minute equilibration period, during which the macula densa was perfused with a low-NaCl solution, the perfusate was switched to high NaCl for 10 minutes and then to low NaCl plus 7-NI (10^{-5} mol/L) for 20 minutes, and finally to high NaCl plus 7-NI for 20 minutes.

Effect of sodium intake on TGF and its potentiation by 7-NI. Young male New Zealand white rabbits (1.2 to 1.5 kg) were given tap water ad libitum and were fed standard rabbit chow that differed only in NaCl content (Ralston Purina, St. Louis, MO, USA) for 3 weeks. The high-, normal- and low-salt diets contained 2.0, 0.2, and 0.05% NaCl, respectively (N = 8, 6, and 7). Plasma renin activity was significantly higher in conscious rabbits fed the low-sodium diet (27.9 ± 5.5 ng angiotensin I/mL/hour) than in those fed a normal-sodium diet (9.5 ± 1.4 ng angiotensin I/mL/hour) or a high-sodium diet (4.8 ± 1.2 ng angiotensin I/mL/hour). After a 20-minute equilibration period, during which the macula densa was perfused with low NaCl, the perfusate was switched to high NaCl for 20 minutes and then to high NaCl plus 7-NI (10^{-5} mol/L) for 20 minutes.

Effect of 7-NI on basal Af-Art diameter. Experiments were performed with JGAs from rabbits fed low, normal or high sodium (N = 14, 14, and 15, respectively). After a

20-minute equilibration period, during which the macula densa was perfused with low NaCl, the perfusate was switched to low NaCl plus 7-NI (10^{-5} mol/L) and the Af-Art was perfused for another 20 minutes.

Effect of adding 7-NI to the Af-Art perfusate. To confirm that 7-NI only inhibits macula densa nNOS and has no direct effect on the Af-Art by inhibiting endothelial nitric oxide synthase (eNOS) in the endothelium, additional experiments were performed in rabbits on normal sodium. When 7-NI (10^{-5} mol/L) was added to the Af-Art perfusate, unlike L-NAME it did not alter basal diameter. The Af-Arts were then preconstricted with norepinephrine (3 to 5×10^{-7} mol/L), and the vasodilator response to acetylcholine (10^{-7}, 10^{-6}, and 10^{-5} mol/L) in the presence of 7-NI (10^{-5} mol/L) was studied.

Statistics

Values are expressed as mean ± SEM. A paired *t* test was used to examine whether the diameter at a given concentration was different from the control value. Analysis of variance was used to examine whether dose-response curves differed between groups, and a two-sample *t* test was used to examine whether changes in diameter at a given concentration differed between groups. $P < 0.05$ was considered significant using Bonferroni's correction for multiple comparisons.

RESULTS

Effect of 7-NI on TGF in nNOS +/+ and −/− mice

Neuronal nitric oxide synthase deficient mice (nNOS −/−) were used to determine whether the NO produced by nNOS in the macula densa modulates the TGF response. We also tested whether 7-NI potentiates TGF response. Figure 1 shows representative findings in nNOS +/+ and −/− mice. TGF response was observed in both strains; however, 7-NI only potentiated the response in nNOS +/+. Figure 2 shows average TGF response in nNOS +/+ mice before and after 7-NI was added to the macula densa perfusate. In the nNOS +/+ mice, changing the macula densa perfusate from low to high NaCl decreased luminal diameter from 14.69 ± 0.77 to 11.96 ± 0.82 μm ($P = 0.002$). When 7-NI was added to the macula densa perfusate, TGF response was significantly potentiated and Af-Art diameter decreased from 14.71 ± 0.92 to 9.51 ± 0.92 μm ($N = 8$, $P = 0.001$ vs. without 7-NI). Figure 3 shows average TGF response in nNOS −/− mice. Af-Art diameter decreased from 15.5 ± 1.06 to 11.81 ± 1.09 μm, similar to the response in nNOS +/+. In contrast, in nNOS −/− mice, adding 7-NI to the macula densa perfusate did not augment TGF (from 15.35 ± 0.76 to 12.02 ± 0.89 μm; $N = 8$; Fig. 3). In time controls, the Af-Art diameter decreased from 14.2 ± 0.9 to 11.8 ± 1.1 μm ($P < 0.05$) when the solution perfusing the macula densa was first changed from low to high NaCl. When the process was repeated, the diameter decreased from 14.2 ± 1.2 to 12.3 ± 0.9 μm ($P < 0.05$). Taken together, these data confirm that chronic changes in macula densa nNOS do not play a major role in the regulation of TGF; however, potentiation of TGF by acute inhibition of nNOS does require macula densa nNOS. These findings also show that 7-NI selectively inhibits nNOS in the macula densa.

Effect of sodium intake on TGF response and its potentiation by 7-NI

Tubuloglomerular feedback responses in JGAs from rabbits on low, normal or high NaCl intake were similar. Adding 7-NI to the high-NaCl macula densa perfusate caused comparable constriction of the Af-Art in all three groups. Diameter decreased by 12.4% in the low-salt group (from 18.7 ± 2.05 to 16.4 ± 2.21 μm, $N = 7$, $P < 0.001$), by 15.0% in the normal-salt group (from 15.4 ± 1.53 to 12.5 ± 1.29 μm, $N = 6$, $P < 0.003$), and by 10.8% in the high-salt group (from 20.7 ± 1.64 to 18.4 ± 1.71 μm, $N = 8$, $P < 0.002$; Figs. 4–6).

Effect of 7-NI on basal Af-Art diameter

Next, we examined the effect of blocking macula densa nNOS when the macula densa was perfused with low NaCl. In JGAs from rabbits fed a low-NaCl diet during perfusion with low NaCl, the Af-Art diameter was 21.7 ± 1.3 μm. Surprisingly, adding 7-NI to the low-NaCl perfusate decreased the diameter by 14%, from 21.7 ± 1.3 to 18.6 ± 1.5 μm ($N = 14$, $P < 0.001$; Fig. 7). In contrast, in JGAs from rabbits on normal or high salt intake, 7-NI did not alter the Af-Art diameter; it was 19.4 ± 0.7 and 19.7 ± 0.8 μm before and after 7-NI ($N = 14$) with normal NaCl and 20.5 ± 0.8 and 20.2 ± 0.8 μm before and after 7-NI with high NaCl ($N = 15$; Fig. 7).

Effect of adding 7-NI to the Af-Art perfusate

In rabbits on normal sodium, Af-Art diameter before and after 7-NI was 20.8 ± 1.1 and 20.7 ± 1.3 μm, respectively ($P > 0.05$, $N = 5$). Norepinephrine decreased Af-Art diameter to 10 ± 2.4 μm. In the presence of 7-NI, acetylcholine at 10^{-7}, 10^{-6}, and 10^{-5} mol/L increased diameter to 13.8 ± 1.3, 17.6 ± 0.9, and 20.0 ± 0.9 μm, respectively.

DISCUSSION

In this study, we found that TGF responses were similar in nNOS +/+ and −/− mice, thus confirming the report of Schnermann et al [12]. However, in nNOS +/+ mice, acute inhibition of macula densa nNOS with 7-NI increased TGF, whereas in nNOS −/− mice this potentiation was not present. We interpreted these results as an indication that NO produced in the macula densa by nNOS regulates TGF acutely but not chronically. Further-

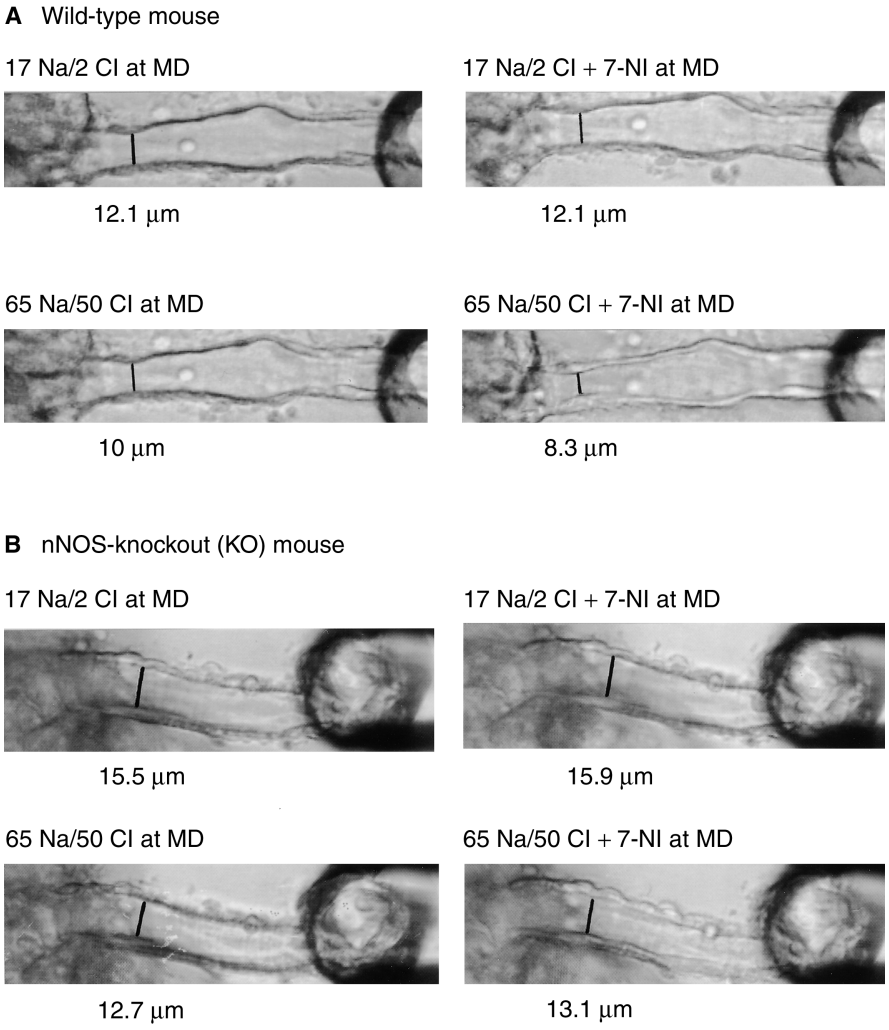

Fig. 1. Afferent arteriole (Af-Art) constriction induced by 7-nitroindazole (7-NI) when the macula densa (MD) was perfused with low or high NaCl in (*A*) wild-type controls (C57BL/6Jx129/SV) and (*B*) neuronal nitric oxide synthase (nNOS) −/− mice. With 65 Na/50 Cl NaCl at the macula densa, 7-NI caused stronger constriction of the Af-Art in wild-type mice than it did in nNOS −/−.

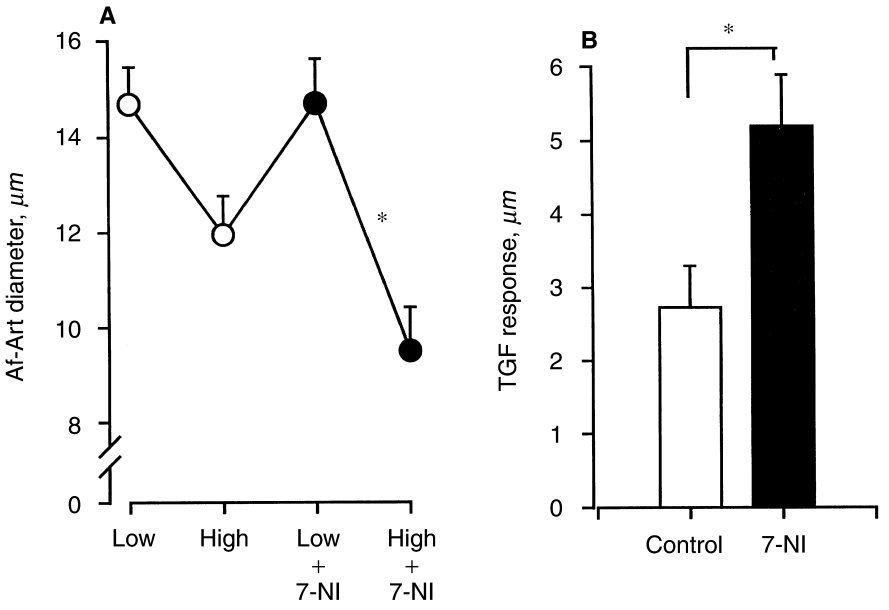

Fig. 2. (*A*) Effect of 7-nitroindazole (7-NI) on afferent arteriole (Af-Art) diameter with different NaCl concentrations at the macula densa in wild-type controls ($N = 8$). The tubuloglomerular feedback (TGF) response was significantly increased with 7-NI. (*B*) Magnitude of changes in the Af-Art during TGF. Symbols are: (□) TGF during perfusion of the macula densa with high NaCl (control); (■) high NaCl plus 7-NI.

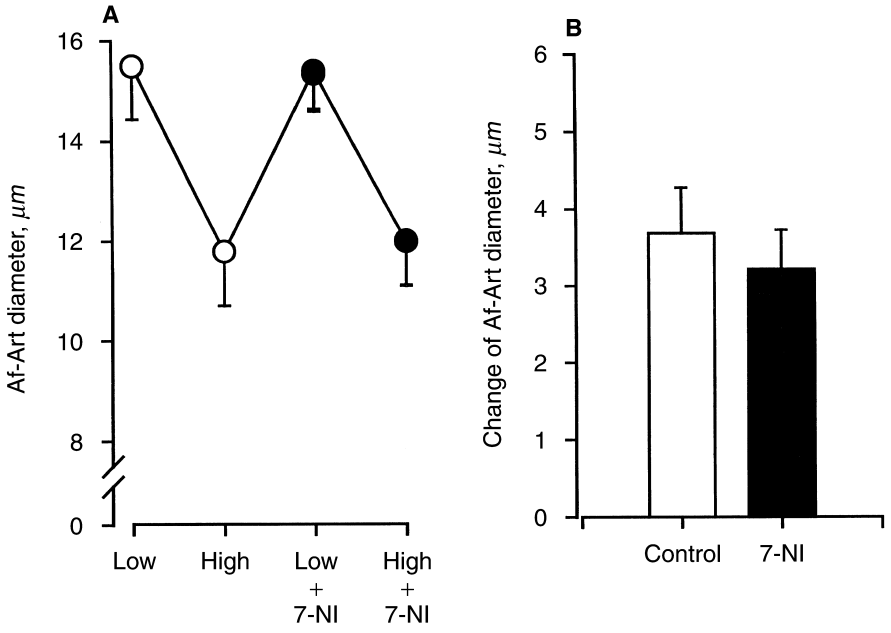

Fig. 3. (*A*) Effect of 7-nitroindazole (7-NI) on afferent arteriole (Af-Art) diameter with different NaCl concentrations at the macula densa in neuronal nitric oxide synthase (nNOS) −/− mice (*N* = 8). (*B*) Magnitude of changes in the Af-Art during tubuloglomerular feedback (TGF). Unlike the wild-type mice, 7-NI did not potentiate TGF. Symbols are: (□) TGF during perfusion of the macula densa with high NaCl (control); (■) high NaCl plus 7-NI.

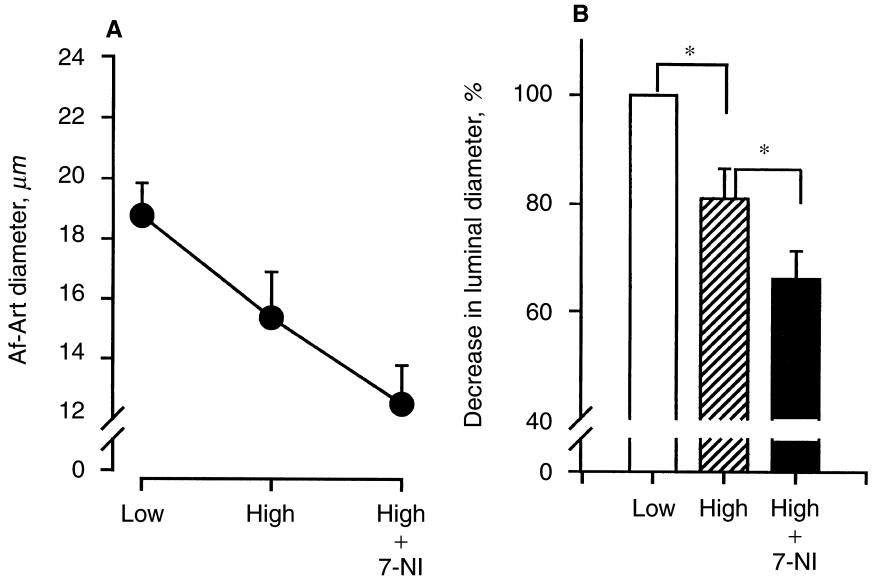

Fig. 4. (*A*) Effect of 7-nitroindazole (7-NI) on afferent arteriole (Af-Art) diameter during perfusion of the macula densa with high NaCl in rabbits on normal sodium intake (*N* = 6). The addition of 7-NI to the high-NaCl perfusate caused further constriction of the Af-Art. (*B*) Changes in Af-Art diameter. During perfusion of the macula densa with low NaCl (□), the diameter was taken as 100%. Symbols are: (▨) changes produced by high NaCl alone; (■) high NaCl plus 7-NI.

more, in JGAs from rabbits on a low-sodium diet (which is reported to increase macula densa nNOS) (abstract; Singh et al, *FASEB J* 9:4888, 1995) [13], TGF responses were similar to those seen with normal or high sodium, likewise suggesting that chronic changes in macula densa nNOS do not play a major role in the regulation of TGF. One possibility is that compensatory mechanisms develop during chronic alteration of nNOS that keep TGF response relatively constant. Also, in rabbits on three different sodium diets, TGF was potentiated equally by 7-NI. Thus, the degree of TGF potentiation appears to be independent of the amount of macula densa nNOS. Potentiation of TGF by 7-NI could be due to its diffusion to the Af-Art—where it may inhibit eNOS—since 7-NI has been reported to inhibit other NOS isoforms [18]. However, our results demonstrate that when 7-NI is administered via the macula densa perfusate, its effect is due to inhibition of nNOS, since TGF was not potentiated in nNOS −/− mice. Furthermore, when 7-NI was added to the Af-Art perfusate, acetylcholine (an endothelium-dependent vasodilator) dilated the arteriole.

We do not have a good explanation as to why TGF in response to an increase in NaCl in the macula densa was not altered by conditions that chronically delete

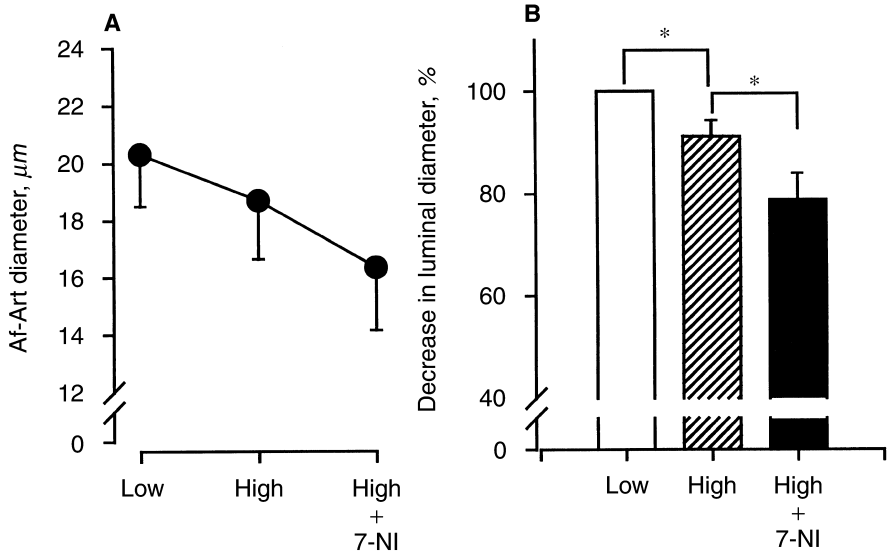

Fig. 5. (*A*) Effect of 7-nitroindazole (7-NI) on afferent arteriole (Af-Art) diameter during perfusion of the macula densa with high NaCl in rabbits on low sodium intake (*N* = 7). The addition of 7-NI to the high-NaCl perfusate caused further constriction of the Af-Art. (*B*) Changes in Af-Art diameter. During perfusion of the macula densa with low NaCl (□), the diameter was taken as 100%. Symbols are: (▨) changes produced by high NaCl alone; (■) high NaCl plus 7-NI.

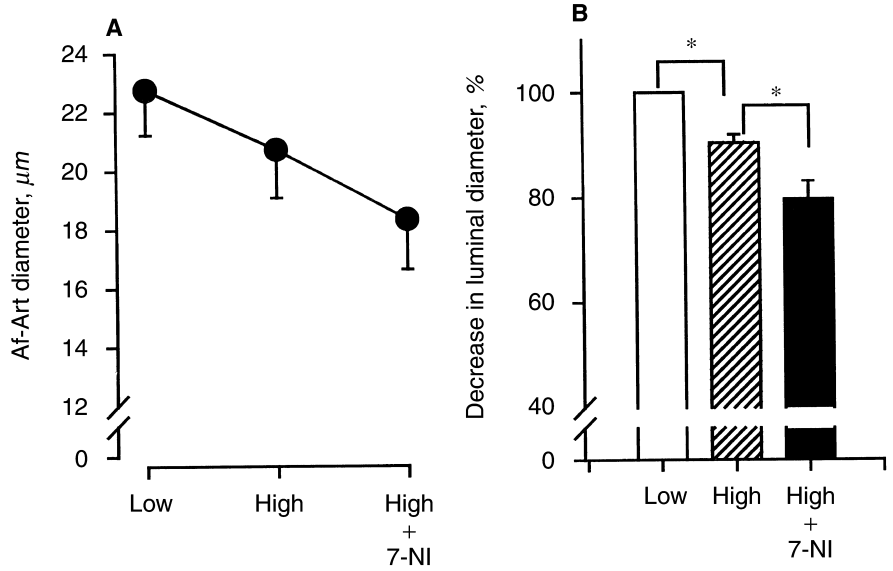

Fig. 6. (*A*) Effect of 7-nitroindazole (7-NI) on afferent arteriole (Af-Art) diameter during perfusion of the macula densa with high NaCl in rabbits on high sodium intake (*N* = 8). The addition of 7-NI to the high-NaCl perfusate caused further constriction of the Af-Art. (*B*) Changes in Af-Art diameter. During perfusion of the macula densa with low NaCl (□), the diameter was taken as 100%. Symbols are: (▨) changes produced by high NaCl alone; (■), high NaCl plus 7-NI.

macula densa nNOS (such as nNOS −/− mice), whereas it increased during the acute blockade of nNOS TGF. Huang et al reported finding residual nNOS activity in the brains of nNOS −/− mice [19]. Brenman et al described two shorter isoforms of nNOS, measuring 136 kD (nNOSβ) and 125 kD (nNOSγ), which exhibited ~80% and ~3%, respectively, of the activity of full-length nNOS [20]. In these shorter isoforms, the second exon, which serves as the homologous recombination target for gene disruption, is spliced out. Thus, Schnermann et al speculated that the shorter isoform of nNOS also may be expressed in the macula densa, explaining why TGF is not increased in nNOS −/− mice [12]. However, our study shows that even if some nNOS does remain in the macula densa, it has no functional importance, since 7-NI did not potentiate TGF in nNOS −/− mice.

In JGAs from rabbits on a low-NaCl diet, adding 7-NI to the macula densa during its perfusion with low NaCl fluid caused Af-Art constriction, decreasing its diameter by 14%. This effect was not observed in JGAs from rabbits fed a normal- or high-NaCl diet. Thus, it could be that during low NaCl intake, NO produced by nNOS in the macula densa is important for regulation of renal blood flow. During low NaCl intake, renin secretion increases, which theoretically should heighten renal vascular resistance, and it could be that the increase in nNOS caused by chronic low NaCl intake via the release of NO antagonizes the vasoconstrictor effect of the renin-

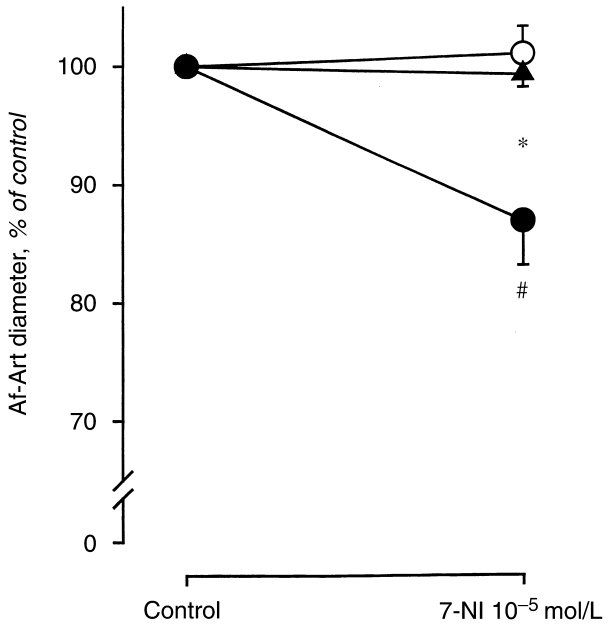

Fig. 7. Effect of 7-nitroindazole (7-NI) on afferent arteriole (Af-Art) diameter during perfusion of the macula densa with low NaCl in rabbits given different sodium diets. In the low-salt group, 7-NI decreased diameter by 14% when the macula densa was perfused with low NaCl ($N = 14$; ●). In contrast, in the high-salt ($N = 15$; ▲) and normal-salt groups ($N = 14$; ○), 7-NI did not alter Af-Art diameter. $*P < 0.05$, low vs. normal- and high-salt intake; $\#P < 0.025$ compared with control.

angiotensin system. This effect appears to be independent of the induction of TGF, since the macula densa was perfused with low sodium. Another possibility is that the threshold of the macula densa NaCl "sensor" decreases during low NaCl intake, thus making the macula densa more sensitive to low Na perfusion.

The presence of nNOS in the macula densa and its acute role in potentiating TGF have been demonstrated by many investigators. Mundel et al reported that nNOS mRNA, immunoreactivity, and enzymatic activity are expressed in the cytoplasm of the macula densa cells [9]. Using in vivo micropuncture, Wilcox et al reported that infusing a nonspecific inhibitor of NOS into the loop of Henle reduced stop-flow pressure, which was prevented by co-infusion of furosemide [8]. This suggests that macula densa NO modulates the TGF response via an effect on furosemide-sensitive sodium transporters. We have previously shown that in our in vitro preparation, NO produced by macula densa nNOS plays a significant role only when TGF is activated [11]. An in vivo study by Brand-Schieber, Pucci, and Nasjletti also supports the role of NO in TGF, since L-NAME-induced renal vasoconstriction was attenuated by furosemide [21]. More recently, Ichihara et al examined the role of macula densa-derived NO in juxtamedullary nephron TGF using a more specific inhibitor of nNOS [5]. They found that when acetazolamide was added to the blood perfusate to increase volume delivery to the macula densa, Af-Art constriction in response to a specific nNOS inhibitor, S-methyl-L-thiocitrulline (L-SMTC), was enhanced, but this effect was completely prevented by papillectomy. Thus, these studies, as well as our own data, clearly show that NO produced by macula densa nNOS either modulates or attenuates TGF.

There is also evidence that the amount of nNOS in the macula densa is altered by salt intake. Using quantitative reverse transcription-polymerase chain reaction (RT-PCR), Singh et al have shown that NOS gene expression in macula densa cells is inversely related to salt intake (abstract; Singh et al, *FASEB J* 9:4888, 1995). Tojo, Madsen, and Wilcox found that immunohistochemical expression of NOS isoforms in the macula densa is increased by dietary salt restriction [13]. Thus, we questioned whether placing rabbits on low sodium would further increase the role of macula densa nNOS in the modulation of TGF. However, despite the fact that nNOS expression is inversely related to sodium intake, we found that Af-Art constriction induced by 7-NI during the TGF response was similar in rabbits on different sodium diets. We had expected that with a low-sodium diet NO would exert a greater influence on modulation of TGF than with a normal diet, and that with high sodium the influence of NO would be even less; however, neither proved to be the case. It could be that in this in vitro preparation the mechanisms that cause resetting of TGF, such as circulating angiotensin II, are absent and thus resetting is not possible. Another possibility is that during the TGF response the amount of nNOS in the macula densa from rabbits on normal and high sodium intake is enough to induce maximal modulation of the acute Af-Art response.

On the other hand, Wilcox and Welch reported that in the rat in vivo microperfusion of the macula densa with L-NMMA elicited greater enhancement of TGF during high salt intake than with low salt [14]. These investigators suggested that changes in macula densa NO generation may be related more to factors that regulate L-arginine delivery and uptake than to the amount of nNOS. There are several differences between the study of Wilcox and Welch and ours that may contribute to the conflicting results. These include the different species used in the two studies, the different levels of sodium intake, and (perhaps most important) the solution they used to perfuse the macula densa did not contain L-arginine, which was present in both the superfusate and the Af-Art medium (MEM; 126 mg/L) in our in vitro preparation. Thus, in our preparation the L-arginine concentrations may have been sufficient to mask any effect of the low-sodium diet on L-arginine delivery and uptake. Since L-arginine transport and plasma concentration both decrease during low sodium intake, lower concentrations of L-arginine may have been a limiting factor in the study of Wilcox and Welch but not in ours. Nevertheless, our

study shows that if TGF is induced by increasing NaCl in the macula densa perfusate and then nNOS is inhibited with 7-NI, the Af-Art constricts further, thus confirming previous results by us and others [7, 8, 22], and further supporting the hypothesis that the release of macula densa NO by nNOS has an inhibitory effect on the Af-Art constriction induced by TGF.

To determine the functional significance of increased macula densa nNOS during low sodium intake, we studied the effect of 7-NI on macula densas perfused with low NaCl (26 mEq), a concentration that causes no changes in Af-Art diameter compared to the diameter with no perfusion of the macula densa (unpublished data). When macula densas were perfused with this NaCl concentration before and during the administration of an nNOS inhibitor, blocking nNOS significantly decreased Af-Art diameter (14%) in JGAs from rabbits on low salt intake but not normal or high salt. We conclude that during low sodium intake, the increased macula densa nNOS results in vasodilation that may oppose the constriction caused by the increased activity of the renin-angiotensin system.

Conclusion

Chronic changes in macula densa nNOS do not play a major role in the regulation of TGF in our in vitro preparation, since in JGAs from mice lacking nNOS, or JGAs from rabbits on a low-sodium diet (which increases macula densa nNOS), the TGF responses were not altered. Compensatory mechanisms may have developed during chronic alteration of nNOS that kept TGF relatively constant. nNOS regulates TGF acutely, since during the TGF response nNOS inhibition caused further constriction of the Af-Art, which was not seen in nNOS −/− mice. Our findings in +/+ and −/− mice also confirm that the effect of 7-NI is due to inhibition of macula densa nNOS. During low sodium intake (without induction of TGF), basal Af-Art resistance was regulated by macula densa nNOS, suggesting that NO in the macula densa helps maintain renal blood flow during the high renin secretion caused by low sodium intake.

ACKNOWLEDGMENT

This study was supported by National Institutes of Health grants HL-28982 and HL-46518.

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