Tubulovascular Nitric Oxide Crosstalk
Buffering of Angiotensin II–Induced Medullary Vasoconstriction

Jeffrey G. Dickhout,* Takefumi Mori,* Allen W. Cowley, Jr

Abstract—Studies were designed to determine the source of NO responsible for buffering of the angiotensin II (Ang II)–mediated decrease of blood flow in the renal medulla. Intracellular Ca$^{2+}$ concentration ([Ca$^{2+}$]$_i$) and NO production ([NO]$_i$) of pericytes and endothelium of the vasa recta were independently measured with the use of fura 2-AM and 4,5-diaminofluorescein diacetate (DAF-2DA), respectively, in microtissue strips of the vascular bundles of the outer medullary vasa recta. Disruption of the endothelium of the vasa recta by perfusion with latex microspheres enabled imaging of the pericytes. Ang II (1 μmol/L) produced an increase of [NO]$_i$ of 19±6 U in pericytes of the vasa recta when the vessels were adjacent to medullary thick ascending limbs (mTALs). Pericytes of isolated vasa recta without surrounding mTALs showed a rapid peak increase in [Ca$^{2+}$]$_i$, of 248±107 nmol/L, with a sustained elevation of 107±75 nmol/L, but did not show an increase in [NO]$_i$ to either Ang II (1 μmol/L) or the Ca$^{2+}$ ionophore 4-bromo-A23187 (5 μmol/L). These observations indicated the lack of Ang II and Ca$^{2+}$-sensitive NO production in pericytes of the vasa recta. In isolated vasa recta with intact endothelium, Ang II reduced [Ca$^{2+}$]$_i$, from 128±28 to 62±13 nmol/L and failed to increase [NO]. However, the Ca$^{2+}$ ionophore did increase [NO]$_i$ in the endothelium (47±8 U), indicating the presence of Ca$^{2+}$-sensitive NO production. Significant increases of [NO]$_i$ were observed in single isolated mTALs in response to both Ang II (33±6 U) and the Ca$^{2+}$ ionophore (51±18 U). We conclude that Ang II increases [Ca$^{2+}$]$_i$ in pericytes of the descending vasa recta as part of its constrictor action and that this vasoconstriction is buffered by the NO from the surrounding tubular elements, such as mTALs. (Circ Res. 2002;91:487-493.)

Key Words: vasa recta ▪ pericytes ▪ endothelium ▪ nitric oxide ▪ intracellular Ca$^{2+}$

Several studies in our laboratory have shown that reductions of renal medullary blood flow caused by angiotensin II (Ang II) are buffered by NO. In anesthetized Sprague-Dawley rats, intravenous infusion of a subpressor dose of Ang II (5 ng/kg per minute) had no effect on medullary or cortical blood flow. However, when this dose of Ang II was given after renal medullary interstitial infusion of a very low dose of Nω-nitro-L-arginine methyl ester (L-NAME), medullary blood flow was significantly depressed, and cortical blood flow was not changed. Microdialysis-oxymoglobin NO trapping in the renal medullary interstitium showed that this dose of Ang II significantly increased interstitial NO concentrations over the drug vehicle by ≈2.5-fold, and this increase in NO was inhibited by the infusion of L-NAME.1 Chronic intravenous infusion of a subpressor dose of Ang II (3 ng/kg per minute) to rats receiving a continuous renal medullary interstitial infusion of L-NAME (at a dose found not to reduce medullary blood flow) resulted in no measurable change of blood flow to the renal cortex but in a significantly depressed medullary blood flow, which led to hypertension over a 5-day period of infusion.2

It has now been shown that medullary blood flow can be regulated independently from cortical blood flow in the kidney; however, the mechanisms involved in the regulation of medullary flow remain to be clarified. The renal outer medulla contains vascular bundles supplied by blood from the juxtamedullary efferent arterioles that divide and form the descending vasa recta, which supply blood to both the outer medullary nephrons and the deepest regions of the inner medulla.4 Outer medullary vascular bundles (OMVBs) of the rat are of the complex type and contain tubular elements from the loops of Henle.5 The vessels of the vasa recta are of two types: arterial (descending) and venous (ascending), with differing distinguishing characteristics. The descending (arterial) vasa recta consist of a continuous layer of endothelium and pericytes occurring at 15- to 20-μm intervals along the vessels. The pericytes possess a round cell body protruding from the vessel with long flattened processes that coil densely around the vessels.6 The ascending (venous) vasa recta are devoid of pericytes and possess a fenestrated endothelium with basal microvilli.6 The pericytes of the descending vasa recta show numerous caveolae and also contain smooth muscle α-actin,7 indicating the presence of a contractile mechanism similar to smooth muscle cells. Pallone8 has shown that isolated perfused descending vasa recta possess...
the ability to constrict to various agonists, including Ang II. Ang II–stimulated constriction of the vasa recta occurred when the drug was added abluminally but occurred to a much lesser extent when given into the lumen.9 These results indicate that the constrictor response of the descending vasa recta produced by Ang II is mediated by stimulation of the pericytes surrounding the outer portions of the vessels.

The present study was designed to clarify the mechanisms whereby Ang II stimulates the production of NO, which, in turn, buffers the reduction of medullary blood flow. We hypothesized that Ang II increases intracellular Ca2+ concentration ([Ca2+]i) as part of its constrictor action in the pericytes of the descending vasa recta and that this constrictor action would be buffered by NO produced either from the endothelial cells of the vasa recta or the tubular epithelial cells surrounding the vasa recta. Ca2+ and NO responses to Ang II in pericytes, endothelial cells, and tubular epithelial cells were selectively measured by using a recently established fluorescence imaging technique for isolated renal microtissue strips.9

**Materials and Methods**

**Experimental Animals**

The present study used male Sprague-Dawley rats weighing 150 to 200 g (Harlan Inc, Madison, Wis) maintained ad libitum on water and a standard pellet diet (Purina Mills) in the Animal Resource Center of the Medical College of Wisconsin. Tissues derived from 5 to 9 animals were used for all protocols.

**Preparation of OMVB Microtissue Strips**

Rats were deeply anesthetized with sodium pentobarbital (50 mg/kg IP), and the kidneys were prepared for dissection, as described in our previous study.9 In addition, some of the kidneys were perfused through the renal artery with 1 mL saline containing a 2.7% (wt/vol) solution of latex microspheres (0.2 μm in diameter, Polysciences) to remove the endothelium of the vasa recta. Microdissection was performed at 4°C under a Leica M3Z stereomicroscope. Four types of microtissue strips were dissected out from the inner stripe of the outer medulla to determine the interaction of Ca2+ and NO: (1) Endothelium-denuded vasa recta with medullary thick ascending limbs (mTALs) were used to study pericytes adjacent to mTALs. (2) Endothelium-denuded isolated single vasa recta without mTALs were used to study the pericyte alone. (3) Intact isolated vasa recta were used to study the endothelial cells. (4) Single isolated mTALs were used to study the epithelial cell responses. Descending vasa recta were readily distinguishable from other segments by the presence of microspheres within the lumen and the rough surface appearance, which was due to the protrusion of pericytes from the outer wall of the vessels. In preparations without microsphere perfusion, descending vasa recta were identified by diameters that were significantly smaller than any other element of the OMVB, by the thickness of the wall by the endothelium, and by the rough surface appearance due to pericytes. The renal tubular element, mTAL, was recognized by a relatively larger diameter and a characteristically thin epithelial wall. Dissected tissues were placed on round glass coverslips coated with the tissue adhesive Cell-tak (BD Biosciences) for fluorescence imaging. Dissection was completed within 60 minutes after the removal of the kidney.

**Fluorescence Detection**

Fluorescence measurements were made by use of a Nikon Diaphot inverted microscope with a ×40 (numerical aperture 0.75) objective. The signal was detected by using a CCD video camera (Hamamatsu Co) coupled to a Gen II image intensifier (Hamamatsu Co). Excitation was provided by a Sutter DG-4 175-W xenon arc lamp (Inovision Co), which allowed high-speed excitation wavelength switching for real-time radiometric measurement of Ca2+. For the experiments, coverslips were placed in an imaging chamber (maintained at 37°C) mounted on the stage of the inverted microscope that allowed the superfusion of the experimental buffer and buffer containing the agonist over the specimens. Regions of the vasa recta or mTAL were selected within the microtissue strips to quantify changes in fluorescence intensity of the signaling dyes with the use of MetaFluor imaging software (Universal Imaging Co).

**Ca2+ Measurements**

The experimental buffer was Hanks’ balanced salt solution (Life Technologies) with 20 mmol/L HEPES adjusted to pH 7.4 (HHBSS buffer), to which 100 μmol/L L-arginine (Sigma Chemical Co) was added. Fura 2-AM (Molecular Probes Inc) was used to measure [Ca2+]i within the various cell types of the OMVB microtissue strips and was loaded into the tissue or renal elements at a concentration of 5 μmol/L in the HHBSS buffer for 30 minutes at room temperature and then washed to remove excess dye. Pluronic F127 (Molecular Probes Inc) was used to dissolve the fura 2-AM dye to prevent dye compartmentalization. The coverslips were then incubated in the experimental buffer for 15 minutes before the experiments. Fura 2 signal fluorescence was stimulated by dual-wavelength excitation (340 nm/380 nm) from the Sutter DG-4 175-W xenon arc lamp and collected through a 510/40-nm bandpass emission filter at 1-second intervals. Fura 2 radiometric responses were calibrated intracellularly by measurement of maximum (ionophores+2.5 mmol/L Ca2+ buffer) and minimum (ionophores+0 mmol/L Ca2+ buffer with 100 μmol/L EGTA) fura responses, as described in our previous study.9

The fura 2 dye was found to load preferentially into the endothelium of the vasa recta as previously noted by Pallone et al.10 However, when perfusion of microspheres was used to disrupt the endothelium of the vasa recta, this signal was abolished. The remaining signal in the pericytes could then be acquired with much higher signal amplification from the Gen II image intensifier without the interference of the brighter endothelial signal. Light amplification with the image intensifier provided a usable fura 2 signal while maintaining rapid acquisition times (1-second signal integration) but degraded image resolution. A Micromax Cooled CCD camera (Princeton Instruments Inc) was used with long on-chip signal integration times to acquire high-resolution white light and fluorescence micrographs of the OMVB to assess dye loading. The appearance of the microsphere-filled descending vasa recta is illustrated in Figure 1; the origin of the fura 2 signal was found to be consistent with pericyte loading.

The Ca2+ signal of the endothelium and pericytes of the descending vasa recta was imaged within the OMVB in response to superfusion of the microtissue strips with the drug vehicle or 1 μmol/L Ang II.
NO production was measured by using the NO-sensitive dye 4,5-diaminofluorescein diacetate (DAF-2DA) under the same experimental conditions as established in our previous study. With these conditions, we have shown the specificity of the DAF-2 dye for NO by abolition of DAF-2 signal increases to the NO-specific donor, DETA-NONOate (Cayman Chemical Co.), in the presence of 2-(4-carboxyphenyl)-4,4',5,5'-tetramethylimidazol-1-oxyl 3-oxide, an NO scavenger, in medullary microtissue strips. In the present study, the specimens were incubated on coverslips in the HBBSS buffer containing 10 μmol/L DAF-2DA (Calbiochem-Novabiochem Co) for 60 minutes at room temperature and then washed to remove excess dye. The tissues were then incubated for 15 minutes in the experimental buffer (HBBSS buffer containing 100 μmol/L L-arginine) before the experiments. DAF-2 was excited at a wavelength of 480 nm and collected through a 535/40-nm bandpass emission filter at 3.5-second intervals. As described in the Ca2+ studies, microsphere infusion was again used to image the pericytes of the vasa recta without the inference of DAF-2 signal from the endothelium. The NO signal of the endothelium and pericytes of the descending vasa recta was imaged within the OMVB in response to superfusion of the microtissue strips with the drug vehicle or 1 μmol/L Ang II. Agonist stimulation was followed by the addition of 20 μmol/L or 1 mmol/L of the NO donor DETA-NONOate as a positive control for dye loading. Vessels that did not respond to positive control stimulation were discarded.

To determine the presence of Ca2+-sensitive NO synthase (NOS) in the various cell types under study, the Ca2+ ionophore 4-bromo-A23187 (Molecular Probes Inc) was used at 5 μmol/L concentration in the experimental buffer to increase [Ca2+], while imaging the DAF-2 NO signal. The NO signals of isolated endothelium and pericytes of the descending vasa recta and of isolated mTALs were imaged in response to superfusion of the specimens with the drug vehicle, 1 μmol/L Ang II or 5 μmol/L 4-bromo-A23187. This allowed determination of whether NO responses from the pericytes of the descending vasa recta were due to NO production within this cell type or due to diffusion of NO to the cells from the surrounding tissue.

The units (U) used to express NO responses are values of DAF-2 signal intensity averaged over regions of interest as they change from basal control states to agonist-stimulated states measured at constant levels of frame integration between experiments. Although the scale of units remains constant, the absolute magnitude of signal is dependent on the intensifier gain that is adjusted according to dye-loading of the specific cell types. Comparisons of the absolute magnitude of response between cell types were therefore not made, and determinations were only expressed as to whether the response did or did not occur in given cell types.

Statistical Analysis

Values are expressed as mean±SE. The time responses of [Ca2+], and intracellular NO production ([NO]) were evaluated with a 1-way ANOVA to determine whether agonist stimulation had produced significant differences from basal levels. If differences from basal levels were found, an unpaired t test was used to determine whether the agonist response was significantly different from the drug vehicle at these specific time points. Significance was accepted at a level of *P<0.05.

Results

NO Response to Ang II From Pericytes of Vasa Recta Adjacent to mTALs in Microtissue Strips

Ang II (1 μmol/L) stimulation of pericytes of the vasa recta adjacent to mTALs produced a slow but significant increase in [NO], from baseline, reaching a maximum at ~100 seconds after stimulation (*P<0.01, n=6; Figure 2A). An average increase in pericyte [NO], of 19±6 U (P<0.05, n=6) was observed 150 seconds after Ang II agonist addition compared with drug vehicle. The NO donor DETA-NONOate (20 μmol/L) produced a significant increase in [NO], (39±12 U) within these pericytes of the vasa recta at 150 seconds (*P<0.05, n=6; Figure 2B).

Ca2+ and NO Responses From Pericytes in Isolated Vasa Recta

To determine whether Ang II could directly increase NO in pericytes and whether this was a Ca2+-mediated pathway, [Ca2+]i and [NO], were monitored in pericytes that did not have surrounding mTALs attached (isolated vasa recta). These vasa recta were also pretreated with microspheres to disrupt the endothelial cells. The addition of Ang II (1 μmol/L) to these pericytes of the vasa recta produced a rapid increase in [Ca2+]i, from a basal level of 113±30 nmol/L to a peak of 364±174 mmol/L at 25 seconds (Figure 3A). The [Ca2+]i values were significantly increased over baseline, as indicted by ANOVA, from 15 to 35 seconds after stimulation (*P<0.05, n=7). The increase tended to be sustained 250 seconds beyond stimulation, averaging 239±110 nmol/L, but it did not reach the level of statistical significance.
In contrast, Ang II (1 μmol/L) stimulation did not significantly change [NO] in the pericytes of the isolated vasa recta. The average change in DAF-2 intensity produced by the drug vehicle (−8±3 U, n=5) and 1 μmol/L Ang II (−10±4 U, n=5) at 150 seconds after stimulation was not found to be significantly different from basal control levels (P=0.90, n=5; Figure 3B). The positive control NO donor, DETA-NONOate (1 mmol/L), significantly increased DAF-2 signal in the pericyte of these vasa recta (79±12 U) at the 150-second time point (P<0.01, n=5). To determine whether Ca2+-sensitive NO production was absent in pericytes, the [NO] response to the Ca2+ ionophore was determined. As also shown in Figure 3B, the Ca2+ ionophore 4-bromo-A23187 (5 μmol/L) also failed to increase [NO], in pericytes (P=0.90, n=5).

Endothelial Ca2+ and NO Responses in Isolated Vasa Recta

To determine whether endothelium of the vasa recta could contribute to the Ang II–stimulated NO increase seen in pericytes, [Ca2+], and [NO], responses to Ang II were determined in endothelium from isolated vasa recta. Ang II (1 μmol/L) stimulation of the endothelium of the vasa recta significantly reduced [Ca2+] from an average baseline concentration of 128±28 nmol/L to a trough of 62±13 nmol/L at 30 to 50 seconds (P<0.01, n=7; Figure 4A). [Ca2+] remained significantly depressed up to 165 seconds after the addition of Ang II and then returned toward the control baseline over the 250-second time course of the experiment. Ang II (1 μmol/L) did not significantly change [NO], in endothelium of the vasa recta from basal levels (Figure 4B). The average changes in DAF-2 intensity produced by the drug vehicle (−8±3 U, n=5) and 1 μmol/L Ang II (−20±9 U) were not significantly different from control, as represented by the average response 150 seconds after stimulation (P=0.22, n=5; Figure 4B). In contrast, the Ca2+ ionophore
significantly increased [NO], in endothelium of the isolated vasa recta (47±8 U, n=5; P<0.01), and the positive control NO donor, DETA-NONOate (1 mmol/L), increased the DAF-2 signal in the endothelium of these vasa recta by 80±17 U, on average, at the 150-second time point (P<0.01, n=5).

**Ca**<sup>2+</sup> and NO Responses From Isolated mTALs

The mTAL was also isolated from the outer medullary bundle of the vasa recta to identify NO production in response to Ang II and to determine the presence of Ca<sup>2+</sup>-sensitive NO production in these epithelial cells. Ang II (1 μmol/L) increased DAF-2 intensity to 33±6 U at 150 seconds, a level significantly greater than that of the drug vehicle (1±3 U) (P<0.01, n=5; Figure 5). The Ca<sup>2+</sup> ionophore also increased [NO], (50±10 U) (P<0.01, n=5) compared with the drug vehicle, and the positive control NO donor, DETA-NONOate (1 mmol/L), significantly increased the DAF-2 signal in the mTAL by 125±42 U, on average, at the 150-second time point (P<0.05, n=5).

**Discussion**

Compared with the cortical circulation, the medullary circulation of the kidney has been found to be more resistant to the vasoconstrictor effects of Ang II. This was demonstrated in anesthetized rats in which intravenous infusion of Ang II (20 ng/kg per minute) resulted in significant reductions in cortical flow (≈20% reduction) without a significant decrease in medullary flow. Reduced Ang II vasoconstrictor responsiveness in the renal medulla appears in large part to be the result of the higher NOS activity found in the renal medulla compared with the renal cortex. Renal medullary interstitial infusion of L-NAME in rats unmask the medullary vasoconstrictor actions of Ang II, whereas even suppressor intravenous doses of Ang II (5 ng/kg per minute) result in significant reductions of medullary blood flow. Moreover, intravenous infusion of Ang II (5 ng/kg per minute) significantly increased medullary interstitial NO concentrations measured by microdialysis-oxyhemoglobin NO trapping. These results have demonstrated the importance of the NO-buffering mechanism in the overall regulation of medullary blood flow.

To determine whether contractile pericytes in OMVBs produce NO intracellularly to buffer vasoconstrictor effects of Ang II, we used the microtissue strip technique that we have recently developed and the fluorescent NO-sensitive indicator DAF-2DA. However, because DAF-2 fluorescence is quite high in the endothelium of the vasa recta, it was not possible to distinguish the pericyte signal from the greater intensity of the endothelial cell signal. To resolve this limitation, microspheres were perfused through the renal artery to disrupt the vascular endothelium, enabling the selective imaging of the pericytes surrounding the vasa recta. As discussed in detail below, these techniques enabled us to demonstrate that the pericytes of the outer medullary vasa recta fail to respond directly to Ang II or to the Ca<sup>2+</sup> ionophore with an increase of intracellular Ca<sup>2+</sup>. The rise of NO seen in these cells in response to Ang II was seen only when the surrounding tubules of the mTALs were present to release NO.

**Pericyte Ca**<sup>2+</sup> and NO Responses to Ang II

The pericytes of the descending vasa recta in the absence of surrounding tubules did not increase [NO], in response to Ang II stimulation. Pericytes were also devoid of Ca<sup>2+</sup>-sensitive NO production, as shown by the absence of an [NO], increase in response to the Ca<sup>2+</sup> ionophore. This indicates that pericytes were not the source of NO buffering for Ang II vasoconstriction. Chakravarthy et al found that retinal capillary pericytes exhibited no immunoreactivity for neuronal or endothelial NOS (eNOS). They also did not exhibit eNOS gene expression as determined by reverse transcription–polymerase chain reaction, and there was no increase in NO production in response to the Ca<sup>2+</sup> ionophore A23187. In contrast, as seen in Figure 3, Ang II did produce immediate increase of intracellular Ca<sup>2+</sup>, a response that could mediate constriction of these vessels of the vasa recta.

**Ca**<sup>2+</sup> and NO Responses to Ang II in Endothelium of Vasa Recta

Ang II failed to increase [NO], in the endothelium of the vasa recta, as shown in Figure 4. This indicates that the source of NO buffering of the Ang II vasoconstrictor effects was not from the endothelium of the vasa recta. However, the Ca<sup>2+</sup> ionophore did increase endothelial [NO], indicating that these endothelial cells do contain a Ca<sup>2+</sup>-sensitive pathway for NO production, although it was not stimulated by Ang II. This agrees with observations that eNOS, mRNA, and enzyme activity is relatively high in the endothelium of the vasa recta, it was not possible to distinguish the pericyte signal from the greater intensity of the endothelial cell signal. To resolve this limitation, microspheres were perfused through the renal artery to disrupt the vascular endothelium, enabling the selective imaging of the pericytes surrounding the vasa recta. As discussed in detail below, these techniques enabled us to demonstrate that the pericytes of the outer medullary vasa recta fail to respond directly to Ang II or to the Ca<sup>2+</sup> ionophore with an increase of intracellular Ca<sup>2+</sup>. The rise of NO seen in these cells in response to Ang II was seen only when the surrounding tubules of the mTALs were present to release NO.
and Ang II type 2 receptors are all present in the vasa recta, as determined by reverse transcription–polymerase chain reaction from microdissected segments. Rhinehart et al showed that the Ang II type 1 receptor antagonist, losartan, prevented the reduction of [Ca\(^{2+}\)], from Ang II. The Ang II type 2 receptor antagonist, PD123319, enhanced the Ang II–inhibitory effect of the [Ca\(^{2+}\)] response by bradykinin and acetylcholine. Furthermore, NOS stimulation with increased [Ca\(^{2+}\)], from the action of bradykinin on the endothelium of the descending vasa recta was found to increase NO production measurable with DAF-2 fluorescence signaling.

Taken together, these observations indicate that Ang II does not directly increase NO in the endothelium of the vasa recta, even though these cells are capable of Ca\(^{2+}\)-mediated NO production. It appears that the Ang II–induced reduction in endothelial [Ca\(^{2+}\)], could serve to actually inhibit NO production (not detectable by DAF-2) and oppose pericyte constriction. However, the results clearly indicate that the vessels of the vasa recta are not the source of NO production responsible for the NO buffering to the constrictor actions of Ang II.

**Evidence of Medullary Tubulovascular Crosstalk to Buffer Reductions of Medullary Blood Flow in the Face of Elevated Levels of Ang II**

Ang II increased [NO], in pericytes only when the vessels of the vasa recta were surrounded by renal tubular segments, such as mTALs. Neither the endothelium nor pericytes could directly increase NO production in response to Ang II. Therefore, we propose that the tubular epithelium of the surrounding tubules is the source of NO for buffering the vasoconstrictor effects of Ang II. Only the mTALs were imaged in the present study, but other tubular elements could also contribute, such as the pars recta of the late proximal tubules or the outer medullary collecting ducts. Ang II clearly increased [NO], in isolated mTALs, and the presence of Ca\(^{2+}\)-sensitive NO production was confirmed in mTALs by stimulation with the Ca\(^{2+}\) ionophore. We cannot exclude the possibility that Ang II could increase NO production in mTALs via eNOS stimulation from a pathway independent of Ca\(^{2+}\)-calmodulin, but this would not appear to be the case for the pericytes because Ang II did not increase [NO], within these cells when they were isolated from surrounding tubules. However, it is possible that Ang II may stimulate the release of substances from mTALs in addition to NO that could act on the pericytes to increase NO through a Ca\(^{2+}\)-independent pathway.

The descending vasa recta form branches that leave the vascular bundles in the inner stripe of the outer medulla and result in a dense capillary plexus. These capillaries supply blood to mTALs at the edges of the OMVBs and to other tubular elements in the interbundle regions. PO\(_2\) in this region of the kidney is relatively low (≈20 to 30 mm Hg), and oxygen delivery in the medulla is limited because of a high rate of metabolic O\(_2\) utilization relative to blood flow. Furthermore, as a consequence of the countercurrent exchange of O\(_2\) between the ascending and descending vasa recta within the outer medulla, mTALs of the interbundle regions operate on the edge of anoxia. Therefore, it is important to maintain medullary perfusion from the descending vasa recta in the face of elevations of Ang II concentration. The importance of this tubulovascular crosstalk was demonstrated by our observations that when medullary NOS activity was reduced by the NOS synthesis inhibitor N\(^6\)-monomethyl-L-arginine, subpressor intravenous infusion of Ang II (5 ng/kg per minute) reduced medullary PO\(_2\) from 23 to 12 mm Hg. This same amount of Ang II did not lower PO\(_2\) under conditions of normal NOS activity. Because the classic role of the mTAL is to reabsorb NaCl and to adjust NaCl delivery for the macula densa, we propose that the mTAL is capable of controlling needed O\(_2\) delivery in the face of high circulating levels of Ang II by diffusing NO to pericytes of the vasa recta.

We conclude that Ang II increases [Ca\(^{2+}\)], in the pericytes of the descending vasa recta as part of its constrictor action on these vessels, as depicted in Figure 6. This contractile action is buffered by NO diffusing from the tubular elements surrounding the bundles of the outer medullary vasa recta, such as mTALs, to the pericytes of the descending vasa recta (tubulovascular crosstalk). The balance between agonist-stimulated vasoconstriction and NO-mediated vasodilation appears to be an important factor in the regulation of medullary blood flow and oxygen supply to the medulla. Therefore, these mechanisms play an important role in the long-term control of arterial blood pressure and tubular homeostasis.

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References


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