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Nitric oxide production by glomerular podocytes

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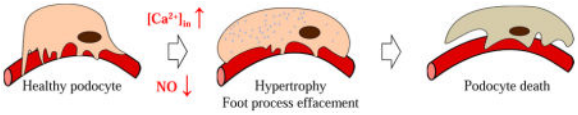
Abstract

Nitric Oxide (NO), a potent vasodilator and vital signaling molecule, has been shown to contribute to the regulation of glomerular ultrafiltration. However, whether changes in NO occur in podocytes during the pathogenesis of salt-sensitive hypertension has not yet been thoroughly examined. We showed here that podocytes produce NO, and further hypothesized that hypertensive animals would exhibit reduced NO production in these cells in response to various paracrine factors, which might contribute to the damage of glomeruli filtration barrier and development of proteinuria. To test this, we isolated glomeruli from the kidneys of Dahl salt-sensitive (SS) rats fed a low salt (LS; 0.4% NaCl) or high salt (HS; 4% NaCl, 3 weeks) diets and loaded podocytes with either a combination of NO and Ca^{2+} fluorophores (DAF-FM and Fura Red, respectively) or DAF-FM alone. Changes in fluorescence were observed with confocal microscopy in response to adenosine triphosphate (ATP), angiotensin II (Ang II), and hydrogen peroxide (H_2O_2). Application of Ang II resulted in activation of both NO and intracellular calcium ($[Ca^{2+}]_i$) transients. In contrast, ATP promoted $[Ca^{2+}]_i$ transients, but did not have any effects on NO production. SS rats fed a HS diet for 3 weeks demonstrated impaired NO production: the response to Ang II or H_2O_2 in podocytes of glomeruli isolated from SS rats fed a HS diet was significantly reduced compared to rats fed a LS diet. Therefore, glomerular podocytes from hypertensive rats showed a diminished NO release in response to Ang II or oxidative stress, suggesting that podocytic NO signaling is dysfunctional in this condition and likely contributes to the development of kidney injury.

Graphical Abstarct



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Conflicts of interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

Keywords

DAF FM; nitric oxide; hypertension; angiotensin II; hydrogen peroxide; Dahl salt-sensitive rat

1. Introduction

Nitric Oxide (NO) is a major signaling molecule in the kidney, where it plays a vital role in the regulation of glomerular filtration. It was reported that deficiency of eNOS (endothelial Nitric Oxide Synthase) results in elevated blood pressure [1], exacerbates renal injury, and accelerates development of proteinuria and glomerulosclerosis [2; 3; 4]. In a mouse model of diabetic nephropathy (DN), absence of eNOS was shown to be critical in the development of kidney injury, and specifically glomerular damage. $eNOS^{-/-}$ mice that were backcrossed to db/db mice exhibited pronounced albuminuria, increased glomerular basement membrane thickness, mesangial expansion, mesangiolysis, and focal segmental and early nodular glomerulosclerosis [5]. From these studies, it seems likely that significant changes in glomerular NO production, which can be triggered by inflammation, oxidative stress or other factors, can lead to glomerular epithelial cell (podocyte) damage and subsequent proteinuria in hypertensive conditions.

The role of reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), superoxide, and NO in the pathophysiology of renal disease, specifically glomerular dysfunction, has been extensively studied in the past [6; 7; 8; 9]. Synthesis of ROS, including NO, in glomeruli can significantly change in various forms of glomerular injury [7; 10; 11; 12]. Oxidative stress due to increased production of ROS in the glomeruli of diabetic and hypertensive rats often correlates directly with increased albuminuria in early stages of these diseases [8; 13]. A recent study by Dolinina et at [14] suggested that the permeability of the glomerular filtration barrier is regulated by a balance between the bioavailability of NO and that of ROS; the authors elegantly showed that in healthy Wistar rats *in vivo* NOS inhibition causes a rapid increase in glomerular vascular permeability, and this phenomenon is dependent upon ROS.

In the kidney, NO can be released by the glomerular and tubular cells in response to a variety of physiological stimuli. It was shown that tubuloglomerular feedback (TGF) mechanisms, triggered by increased tubular perfusion, results in NO production by the macula densa cells [15]. Shear stress [16], endothelin-1 [17; 18], and insulin [19] can stimulate NO generation in the collecting duct. Moreover, increased Na^+ concentration or luminal flow [20; 21] and angiotensin II (Ang II) [22] can increase NO production in the thick ascending limb. Ang II also stimulates release of NO in afferent arterioles [23]. Multiple studies have shown that NO level in the renal microvasculature affects glomerular permeability: for instance, there is greater abundance of NOS at the efferent versus afferent arterioles [24]; thus, reduced NO at the efferent endothelium may predispose to higher levels of glomerular capillary pressure, podocyte injury and glomerulosclerosis. Furthermore, a more recent study featuring microCT-based analyses of vasculature in the renal cortex revealed a loss of perfusable arterioles and glomeruli in $eNOS^{-/-}$ mice [25]. Altogether, the release of NO from the endothelium plays a crucial role in the maintenance of normal barrier permeability, and

these effects should always be taken into considerations when studying the glomerulus as a whole [26].

Extracellular nucleotides stimulate an increase in glomerular albumin permeability due, in part, to glomerular NO production and actin reorganization in podocytes [27]. It was also shown that adenosine triphosphate (ATP) stimulates NO production in a number of cell types including endothelial cells [28], which play a crucial role in the TGF mechanism [29]. Furthermore, we and others previously reported that purinergic signaling is directly involved in the regulation of intracellular calcium ($[Ca^{2+}]_i$) in podocytes [30; 31] and glomerular albumin permeability [27], which can ultimately have effects on eNOS activation. Collectively, these studies suggest a complex regulatory network of NO and glomerular function.

Interdependent regulation of NO and calcium signaling is an important mechanism, which plays a critical role in many cell types. The majority of data for NO-Ca^{2+} interaction in the renal tissue is reported in the vasculature. Thus, recent reports showed that in skeletal muscle arterioles chronic NO deficit induced by L-NAME treatment results in the upregulation of T-type calcium channels [32; 33]. In vascular endothelial cells regulation of eNOS was reported to be calcium-dependent [34]. Additionally, stimulation of calcium-sensing receptors in vascular smooth muscle cells was shown to induce endothelium-dependent vasorelaxation, in part via a pathway involving production of NO, which resulted in activation of BK (large Ca^{2+}-activated K^+) channels [35]. Calcium-NO interaction in other cell types rather than vascular is less studied.

Yuen *et al.* performed an elegant study where they addressed the role of eNOS in podocytes of a diabetic model. Importantly, the authors noted that the development of acute hyperglycemia in $eNOS^{-/-}$ mice leads to podocyte injury, which could be prevented by inhibiting the Renin-Angiotensin-Aldosterone System (RAAS) with captopril or losartan [36]. These findings suggest that NO-mediated podocytopathy may be caused by paracrine (and possibly autocrine) mechanisms, and that RAAS plays an important role in this pathology. Considering the contribution of NO signaling in blood pressure control [1], identification of the factors causing podocyte dysfunction can reveal novel insight into the pathogenesis of hypertension. We propose that activation of specific signaling pathways result in changes in $[Ca^{2+}]_i$ as well as generation of NO by podocytes. We further hypothesized that various paracrine factors compromised during salt-sensitive hypertension can trigger reduced NO production in podocytes and potentially contribute to damage of these cells.

2. Materials and Methods

2.1. Animals

Animal use and welfare adhered to the NIH Guide for the Care and Use of Laboratory Animals following a protocol reviewed and approved by the IACUC of the Medical College of Wisconsin. Either 8 (for the protocol with diet change) or 12 weeks old male Dahl salt-sensitive (SS) rats (SS/JrHsdMcwi) were used for experiments. Water was provided *ad libitum* and the salt content of each diet was 0.4% NaCl AIN-76 diet (#113755; Dyets,

Bethlehem, PA) from weaning up to 8 weeks of age. At 8 weeks of age, the salt content of the chow was either maintained at 0.4% NaCl in the group fed a normal diet or increased to 4.0% NaCl AIN-76 diet (#113756; Dyets) and the rats were maintained on these diets for additional 3 weeks as previously described [37].

2.2. Isolation of the rat glomeruli and intracellular calcium and NO imaging

Experimental procedures were performed as described in our earlier publications [38; 39; 40]. Briefly, kidneys of 12 weeks old SS rats were cleared from the blood, excised and decapsulated; the cortex was isolated and minced using a singled edge razor blade. The minced tissue was sequentially pushed through a steel 100 mesh sieve and then pipetted through a 140 mesh sieve (04-881-5Z and 04-881-5X; Fisher Sci) using the culture medium solution RPMI1640 (Invitrogen, Inc) with 5% BSA. This tissue homogenate was then pipetted onto a 200 mesh sieve (S4145; Sigma) leaving the glomeruli on the top surface. The glomeruli were rinsed using the RPMI-BSA solution into a 15 ml conical tube and settled on ice. After sedimentation the excess of RPMI storage solution was removed and the isolated decapsulated glomeruli were used for confocal microscopy experiments. Isolated glomeruli were allowed to adhere onto 5 × 5 mm coverglass coated with poly-L-lysine (P4707; Sigma). Glomeruli were subjected to confocal measurements immediately after preparations in solution containing (in mM): 145 NaCl, 4.5 KCl, 2 $CaCl_2$, 2 $MgCl_2$, 10 HEPES, pH 7.35). Fluorescence intensities were monitored by confocal laser scanning microscope system Nikon A1-R, detected using an oil immersed Plan Apo 60x/NA 1.4 Oil objective (Nikon) with the argon laser synchronous excitation at 488 nm and selective transmission of light trough the emission filters 520/25 and 680/25 for 4-amino-5 methylamino-2′,7′-difluorescein (DAF-FM, 5 μM; Cat. #D23844, Invitrogen) and Fura Red (2.5 μM; Cat. #F3021, Invitrogen), respectively. Fluorescence images were collected with 0.25 Hz frequency and processed with open source software Fiji (ImageJ 1.47v, National Institute of Health, USA).

2.3. Glomerular volume dynamics assay

For the measurements of the glomerular volume changes Dahl SS rats kidneys were harvested as described earlier, then glomeruli were isolated by differential sieving and stored on ice in a 5% BSA/RPMI solution with glomeruli non-permeable 150 kDa TRITC labeled dextran (TdB Consultancy AB, Uppsala, Sweden) as recently described [41; 42]. Then, glomeruli were attached to poly-L-lysine covered glass coverslips for imaging. Fluorescence intensity detected with the TRITC filter were monitored by confocal laser scanning microscope system Nikon A1-R, and represented the outer glomeruli space. A Z-stack of 27 consecutive focal planes (total slice thickness of ~72.5 μm) was collected every 2 minutes, which allowed reconstructing glomeruli volume using Fiji image processing package (ImageJ 1.47v, National Institute of Health, USA) and Origin Pro 9.0 (OriginLab, Northampton, MA). Volume changes created by the oncotic pressure induced by switching the surrounding medium from 5% into 1% BSA (dialyzed overnight through 50kDa filter) was monitored by 3D imaging throughout the experiment. For glomeruli volume reconstitution, TRITC signal was inverted, and each focal plane area (0.7984 pixel/micron) was processed by the Analyze Particles module (Fiji). Finally, glomerular volume was

calculated by the integration of the obtained focal planes using OriginPro software. The details of this specific approach can be found in our recent publications [41; 42].

2.4. Statistical analysis

Data are presented as mean ± SEM. The values of intracellular calcium ion or NO production concentration at every moment of time for individual cells were averaged by the number of regions registered in the experiment (n = 15–20). Data were compared using the one-way ANOVA, and $P < 0.05$ is considered significant.

3. Results

3.1. Loading of glomeruli

We have previously described an approach that can be used to monitor $[Ca^{2+}]_i$ level in freshly isolated murine glomeruli [38]. Here we applied a similar technique, in which isolated decapsulated glomeruli were loaded with a calcium dye, Fura Red. In addition to $[Ca^{2+}]_i$ measurements, we used the fluorescent indicator DAF-FM to detect NO production, either separately or together with Fura Red. Shown in Fig. 1 is a simplified schematic of the protocol and representative images of an isolated glomerulus loaded with DAF-FM and Fura Red.

3.2. Ang II and H_2O_2 evoke NO production in podocytes of isolated glomeruli

Our previous studies revealed that Ang II and ATP have profound effects on podocyte $[Ca^{2+}]_i$ levels [30; 39; 40]. Since NO-Ca^{2+} interaction is established, we tested the effects of Ang II and ATP on NO production and $[Ca^{2+}]_i$ transients in glomerular podocytes. To do this, confocal fluorescence imaging was done on isolated and decapsulated glomeruli from SS rats loaded with two dyes: DAF-FM for the detection of NO production and Fura Red for monitoring $[Ca^{2+}]_i$ transients. Both Ang II and ATP stimulated $[Ca^{2+}]_i$ release in glomeruli podocytes consistent with previous results [30; 39; 40] (Fig. 2A). However, only Ang II caused a significant increase in production of NO in these cells (Fig. 2B).

We further characterized the effects of Ang II and H_2O_2 on NO production. In these experiments, we used L-NAME to inhibit NOS in order to demonstrate the specificity and sensitivity of the detected response. Shown in Fig. 3A are representative images of an isolated glomerulus loaded with DAF-FM before and after application of Ang II. As summarized in Fig. 3B, Ang II acutely stimulated NO production in this preparation. Addition of L-NAME post-Ang II treatment caused a slight reduction in NO (top graph), whereas pretreatment with L-NAME almost completely abolished the effect of Ang II (bottom graph). Application of H_2O_2 similarly resulted in an immediate elevation of NO levels that was mostly abolished when glomeruli were pretreated with L-NAME (Fig. 3C). It should be noted that the presence of both an intracellular NO source and H_2O_2 may overestimate the level of NO production due to the DAF-FM dye properties, as previously described [43]. However, the observed changes in fluorescence are likely accurate similar to previous reports [44], since this effect was dramatically decreased by pretreatment with L-NAME (Fig. 3C).

3.3. NO production in podocytes in response to Ang II and H_2O_2 upon development of salt-sensitive hypertension

Experiments shown in Figs. 2 and 3 were performed using glomeruli isolated from SS rats fed a 0.4% NaCl diet. Following studies were designed to determine whether NO production in response to these stimuli was altered under hypertensive conditions. To test this, we took advantage of the Dahl SS hypertensive rat model, which is a well-characterized model of salt-induced hypertension [37; 45; 46; 47]. Importantly, SS rats fed a high salt diet develop kidney injury that mimics conditions observed in human salt-sensitive hypertension, including focal segmental glomerulosclerosis (FSGS) [48]. Glomeruli were isolated from the SS rats fed either a low salt (0.4% NaCl) of high salt (HS; 4% NaCl, 3 weeks) diet. As shown in Fig. 3, addition of Ang II and H_2O_2 caused a rapid increase of NO level in glomeruli isolated from rats fed a low salt diet. In rats fed a HS diet for 3 weeks, responses to Ang II- and H_2O_2 were significantly attenuated indicating that NO signaling pathways were disrupted during hypertensive conditions (Fig. 4A and 4B).

3.4. The effects of NO donor on glomerular volume dynamics and permeability in the Dahl SS rats

To further test the effects of NO bioavailability on the changes in glomerular filtration barrier permeability we conducted experiments designed to detect glomerular volume dynamics in response to changes in oncotic pressure (technique initially introduced by Savin *et al.* [49] and adapted by us in our recent manuscript [41]). Freshly isolated glomeruli were pretreated for 40 min with NO donor - DETA NONOate (50 μM) and placed in bath solution used for NO imaging in podocytes. Fast *xyzt* confocal scan was applied before and after introducing colloid osmotic pressure (exerted by the changes in bath solution albumin concentration from 5% to 1%). Obtained confocal images of glomerular profiles at certain Z-positions were recalculated into volume values (see Fig. 5); final mean glomerular volume changes were 112.4±1.9 vs 104.1±0.9 % for control and DETA NONOate pretreated groups, correspondingly. This suggests that the albumin reflection coefficient was lower (indicating greater permeability) when NO bioavailability is increased.

4. Discussion

NO is a major paracrine and autocrine signaling molecule. In the kidney, it can be released by the glomerular and tubular cells, and it is also known to regulate renal hemodynamics and other mechanisms. Changes in glomerular NO production, which can be triggered by activation of RAAS, oxidative stress or other factors, can lead to damage of podocytes along with endothelial and tubular cells, and cause subsequent alterations in glomerular filtration rate resulting in severe proteinuria. For, instance, Li *et al.* demonstrated that exposure of isolated glomeruli to NO donors SNAP, DETA-NONOate and sodium nitroprusside resulted in compromised integrity of the glomerular filtration barrier and increased albumin permeability [50]. The importance of NO role in the regulation of renal microvasculature function cannot be overemphasized: multiple studies of vascular barrier function over the years have clearly demonstrated an increase in vascular permeability after NOS inhibition [51], which could be reversed by introducing NO donors into the system [52; 53; 54]. However, as stated in the recent manuscript by Dolinina *et al.* [14], which studied the role of

NO-ROS antagonism in glomerular permeability and focused majorly on the effects this process has on microvasculature, "*podocytes are important for the barrier function … through their interactions with the rest of the GFB, …, and modify its function without gross changes in cell shape*". Therefore, in this study we focused primarily on the podocyte as the source and effector of NO production.

The ability to detect NO production in podocytes upon activation of specific signaling pathways is important for studying glomerular disease pathogenesis. There are several approaches to measure the concentrations of ROS, including NO, in cells, tissues, and biological fluids [55]. Regarded as an acceptable fluorophore for measuring NO, the aromatic vicinal diamines of diaminofluoresceins (DAFs) have been designed to react with NO in the presence of dioxygen [56]. The large Stokes shift of a calcium dye Fura Red allows for the ability to measure many emission spectra using a single excitation wavelength [57]; this property allowed us to simultaneously measure DAF-FM and Fura Red in response to application of either ATP or Ang II. Combining these two fluorescent probes can provide insight into the cell signaling pathways or processes that may be occurring concurrently. As an example, similar approach has been shown to be useful for detecting NO with DAF-FM-DA probe in combination with a CellTracker Red CMTPX probe [58].

Currently the interest in the interdependent regulation of NO and calcium signaling in various types of cells and tissues is rapidly growing. A recent report by Kim *et al.* [59] demonstrated an indirect correlation between the levels of NO and calcium ions in the kidney using a dual microsensor inserted in a living rat kidney. The crosstalk between NO and calcium signaling is very complex; according to existing reports, NO is able to both induce or reduce $[Ca^{2+}]_i$ concentration, and exert its action directly or indirectly, depending on the cell type or pathophysiological context [60]. Many calcium channels were shown to be regulated by NO either indirectly, when NO could modulate the channels via the generation of cGMP, cyclic ADP ribose, and/or induce protein kinases or directly, in which case NO might regulate channel activity through a post-translational modification (S-nitrosylation) [61; 62; 63]. Additionally, NO can affect Ca^{2+} pumps, including those located in sarcoplasmic/endoplasmic reticulum and plasma membrane, which results in cytosolic Ca^{2+}-removal [64]. An interesting recent study reported that blockade of calcium channels or Ang II type 1 receptors (AT_1R) in humans opposed the renal effects of NOS inhibition by counteracting oxidative stress responses to acutely impaired renal NO bioavailability [65].

Our results indicated that application of Ang II and H_2O_2 caused a rapid increase in NO generation in glomeruli podocytes. Interestingly, ATP had a profound effect on $[Ca^{2+}]_i$ but did not elicit any changes in NO production. It was reported previously that eNOS can be regulated independently from $[Ca^{2+}]_i$ but it usually results in low NO production [66]. This was likely the case in our experiments, and we suggest that the increase in $[Ca^{2+}]_i$ in response to ATP by itself was insufficient to cause changes in NO. Previous studies by Jankowski and colleagues revealed that extracellular ATP may increase the filtration surface of the glomeruli via activation of P2Y receptors with the subsequent activation of the eNOS and soluble guanylyl cyclase [67] and that extracellular nucleotides stimulate an increase of glomerular albumin permeability via NOS pathway [27]. We were not able to detect any effects of ATP on NO production in the podocytes in our preparation. It is likely, however,

that ATP and other purines mediate their effect on glomerular permeability via effects on other glomerular cells, or NO generation, for this process, simply requires more time. The current study focused on the role of NO production in the podocytes in response to Ang II, a peptide hormone of the RAAS.

The role of NO and its regulation by Ang II in salt-sensitive hypertension has been well described [68; 69; 70]. For instance, it was shown in normotensive humans who consumed a high salt diet, as well as in salt-sensitive individuals, that salt sensitivity could be mediated, in part, via a reduction in NO bioavailability [71; 72]. It was also reported that podocyte dysfunction and the development of proteinuria could be associated with a NO deficiency [73]. Genome-wide association study identified a hypertension susceptibility locus located in the promoter region of the eNOS gene [74]. For our experiments we used the Dahl SS rat, a naturally occurring model of salt-sensitive hypertension, which recapitulates many aspects of progressive human hypertension and has provided crucial insights into the mechanisms underlying salt sensitivity and renal injury [37; 75; 76; 77]. This model was used in previous studies revealing the contribution of NO to the development of salt-induced hypertension. About 25 years ago Chen and Sanders published a seminal study showing that L-arginine, the substrate for NO synthesis, abrogates salt-sensitive hypertension in Dahl SS rats [78]. Several groups further confirmed this important observation [79; 80; 81; 82; 83; 84]. Clinical studies also revealed that L-arginine infusion lowered blood pressure in salt-sensitive patients [85; 86]. As summarized in a recent review by Feng *et al.*, endothelial responses to high salt intake affected arteriolar vasodilation and blood pressure [69]. Consistent with this, it was shown that glomerular production of NO is enhanced by increasing dietary sodium and it was proposed that endothelial NOS mediates this response [87]. Most of these studies have focused on the endothelial-podocyte crosstalk, suggesting that endothelial injury contributes to the development of kidney diseases. Our experiments revealed that podocytes themselves are able to produce NO, and that the Dahl SS hypertensive rat exhibit impaired podocyte NO production in response to Ang II and H_2O_2. Our data emphasizes the potential effects and importance of NO generation by podocytes and its necessary role in regulating renal function.

Interestingly, rapid increase in NO bioavailability in whole glomeruli introduced by an NO donor resulted in compromised integrity of glomeruli filtration barrier and an increase in permeability; on the other hand, NO production in podocytes was impaired with the development of hypertension. These effects suggest that podocytes are presumably working to compensate reduction in glomerular permeability by decreasing NO production during the development of hypertension. However, such compensatory action may be not enough to protect podocytes from yet other damage, such as that mediated by the $[Ca^{2+}]_i$ increase and consequent cell apoptosis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- Application of Ang II results in a rapid elevation of NO and $[Ca^{2+}]_i$ in the podocytes.
- Effect of Ang II on NO production is specific, since ATP, a well-known activator of $[Ca^{2+}]_i$ signaling, does not affect NO level.
- Dahl SS rats fed a HS diet for the 3 weeks demonstrated impaired NO production in response to Ang II.

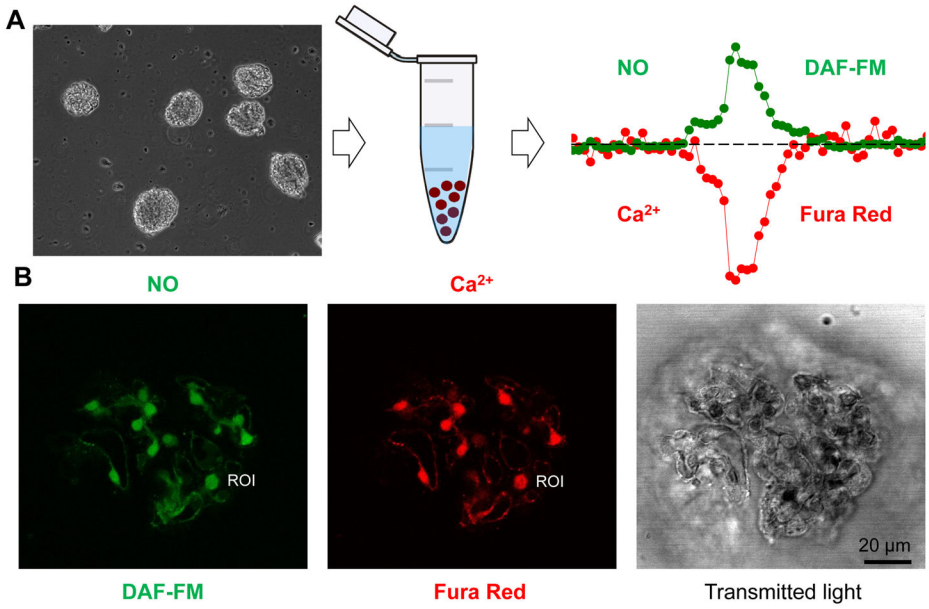

Fig. 1.

Method of glomeruli isolation and confocal imaging. (A) The kidneys are excised and glomeruli are isolated from renal cortex by differential sieving as reported previously [38; 49]. Shown is an image of freshly isolated decapsulated glomeruli, which are subsequently loaded with fluorescent calcium (Fura Red) and NO (DAF-FM) dyes to perform confocal imaging. Scale bar is 80 μm. (B) Representative images of a rat glomerulus stained with DAF-FM (green pseudocolor) and Fura Red (red pseudocolor). Image of the same glomerulus taken with transmitted light is also shown. ROI – single Region of Interest (one podocyte) used for analysis.

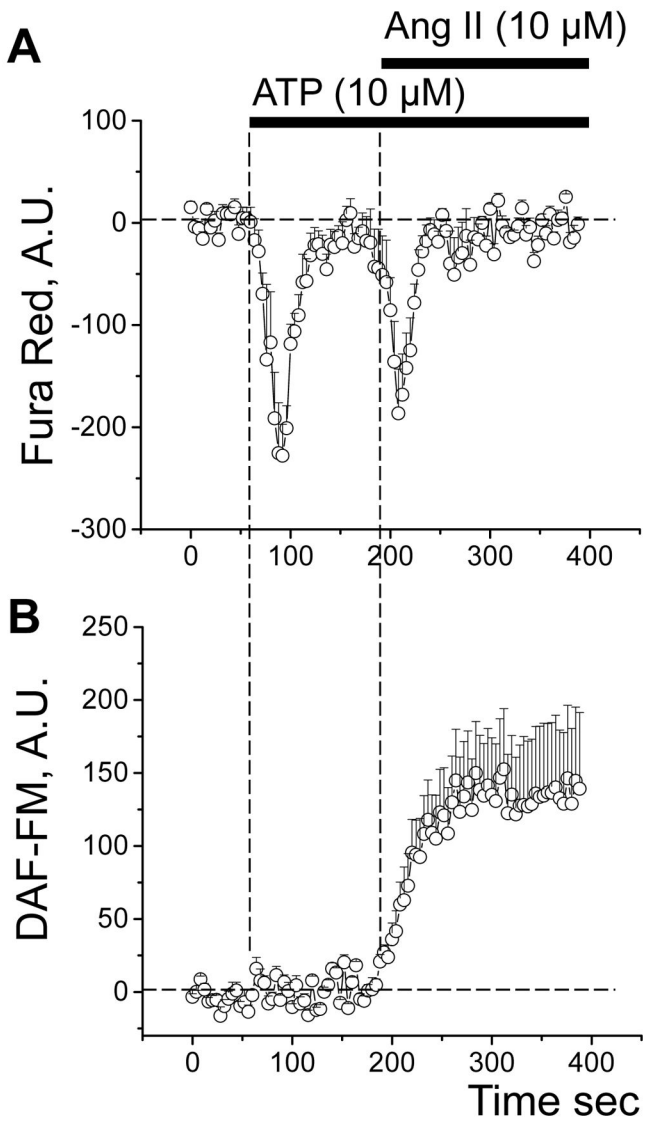

Fig. 2.

Ang II evokes NO production and an increase in $[Ca^{2+}]_i$ in glomerular podocytes of SS rats. (A) Representative transients of $[Ca^{2+}]_i$ dynamics in the podocytes of the Fura Red loaded SS rat glomeruli in response to application of ATP and Ang II. (B) DAF-FM transient increase demonstrating NO production in response to the same agents. ATP application produces a Ca^{2+} transient without affecting NO production in glomeruli podocytes. In contrast, application of Ang II promotes both Ca^{2+} and NO level elevation (note that a decrease in Fura Red signal shows elevation of intracellular Ca^{2+}).

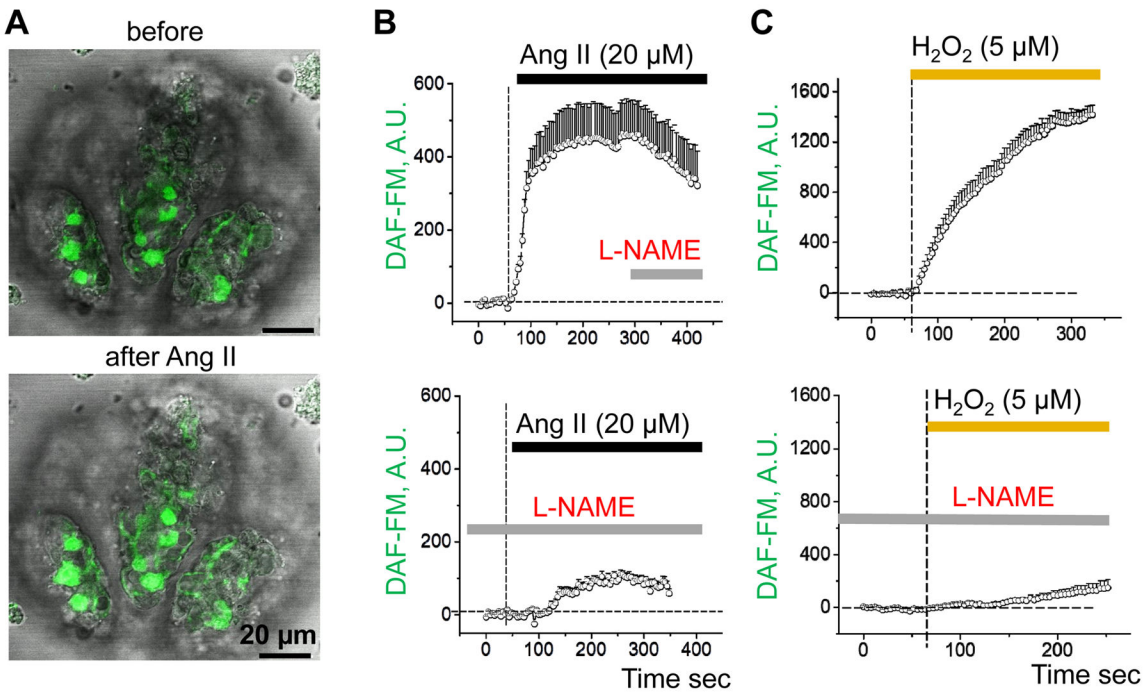

Fig. 3.

Ang II and H_2O_2 evoke NO production in glomeruli podocytes. (A) Confocal imaging of glomeruli before (upper panel) and after (lower panel) Ang II application (merged with transmitted light). Scale bars are shown. DAF-FM transient increase demonstrating elevated levels of NO in response to 20 μM Ang II (B) or 5 μM H_2O_2 (C). Both Ang II- and H_2O_2-mediated NO production significantly decreased after pre-treatment of glomeruli with L-NAME (10 mM).

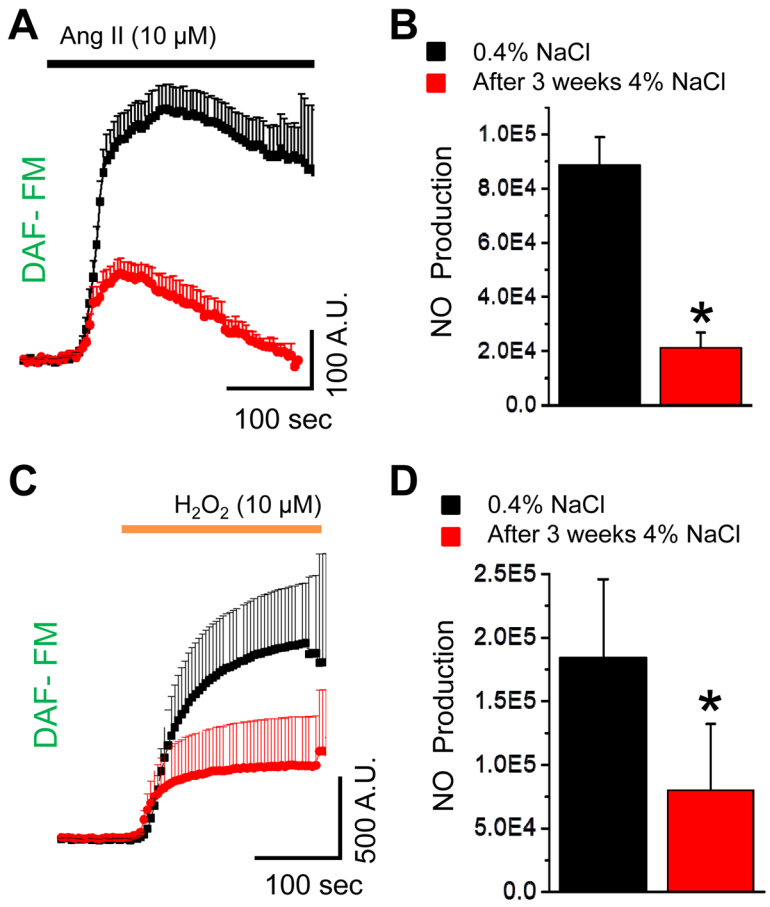

Fig. 4.

Impaired NO signaling in glomeruli podocytes of SS rats upon the development of salt-sensitive hypertension. (A) Changes in podocytes DAF-FM fluorescence in response to Ang II (10 μM) application on glomeruli of SS rats fed a LS (0.4% NaCl; black) and HS (4% NaCl, 3 weeks; red) diets. (B) Total NO production in response to Ang II stimulation calculated as an integral of DAF-FM transient for the 300 sec time interval (N≥5; n≥38 for each group; *P<0.05). (C) Changes in podocytes DAF-FM fluorescence in response to H_2O_2 (10 μM) in SS rats fed LS or HS diets. (D) Graph summarizing changes in H_2O_2-mediated NO production in glomeruli podocytes (N≥4; n≥30 for each group; *P<0.05).

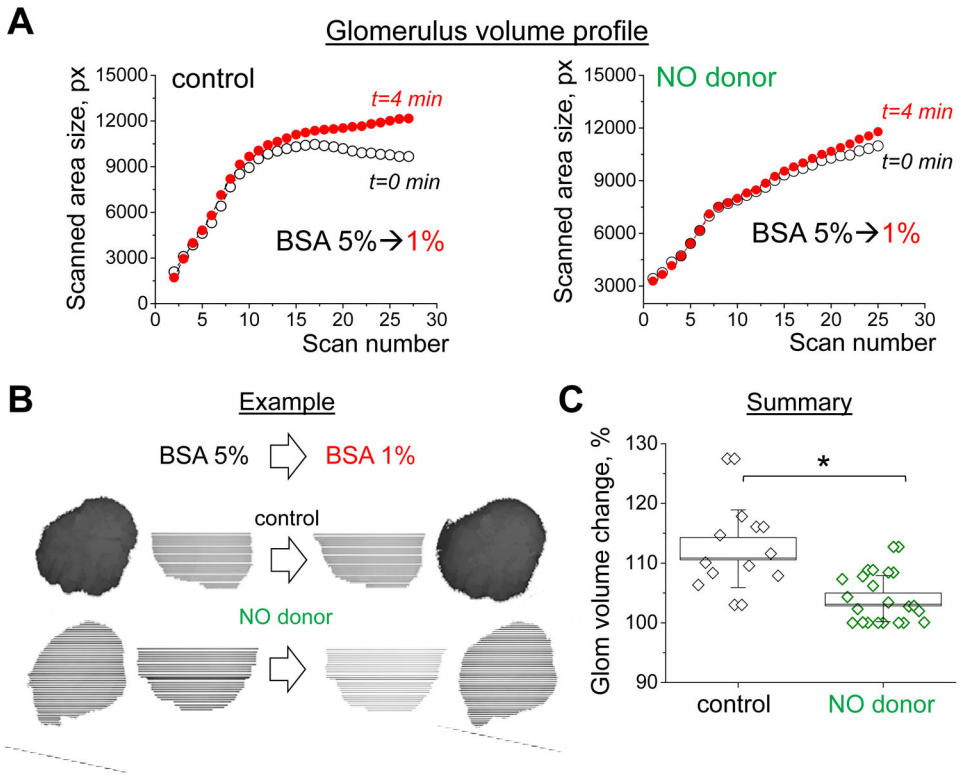

Fig. 5.

Changes in glomerular volume and permeability during the activation of NO signaling cascades in freshly isolated rat glomeruli. (A) Glomerular volume dynamics in response to changes in osmotic pressure (BSA changes from 5 to 1%) in control (left) or DETA NONOate pretreated (right) glomeruli. The line/symbol represents glomerular volume profile (each symbol is the size of a certain z-scan) before (black; t=0) and after (red; t=4min) change in osmotic pressure. (B) An example of glomerular volume change observed by confocal microscopy (shown are images in 5% BSA and after solution change to 1% BSA). (C) A summary of glomerular volumes (which reversibly correlates with the permeability of glomeruli filtration barrier) in control (vehicle) and DETA NONOate pretreated groups (N≥4; n≥12 for each group; *P<0.05).