Distinct Role of Nitric Oxide and Endothelium-Derived Hyperpolarizing Factor in Renal Microcirculation

Studies in the Isolated Perfused Hydronephrotic Kidney

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Key Words

Nitric oxide • Endothelium-derived hyperpolarizing factor • Afferent arteriole • Interlobular artery • Renal microcirculation

Abstract

Background: Both nitric oxide (NO) and endothelium-derived hyperpolarizing factor (EDHF) are established as important factors determining the vascular tone. The relative contribution of these factors to the renal microvascular tone, however, has not been delineated. **Methods:** Isolated perfused hydronephrotic rat kidneys were used to characterize the relative role of NO and EDHF in mediating the tone of interlobular arteries (ILA) and afferent arterioles (AFF). **Results:** During the norepinephrine constriction, acetylcholine (ACH, 1 $\mu\text{mol/l}$) induced a sustained vasodilation of ILA ($90 \pm 9\%$ reversal) and AFF ($117 \pm 13\%$ reversal). In the presence of nitro-L-arginine methylester (LNAME), the ACH-induced vasodilation of ILA and AFF was converted to transient dilation, with only 53 ± 7 and $32 \pm 7\%$ reversal observed 10 min after 1 $\mu\text{mol/l}$ ACH (i.e. sustained phase). In contrast, LNAME had no effect on the initial phase of ACH-induced dila-

tion. In the presence of apamin + charybdotoxin, the initial vasodilator response to ACH (1 $\mu\text{mol/l}$) was diminished (ILA, from 108 ± 8 to $46 \pm 9\%$; AFF, from 108 ± 14 to $58 \pm 8\%$), whereas no impairment was observed in sustained phases. Furthermore, the magnitude of the vasoconstriction caused by LNAME was greater at smaller vessel segments. Finally, the LNAME-induced inhibition of the sustained phase of ACH-induced vasodilation was greater as the vessel diameter decreased. **Conclusions:** That the relative contribution of NO and EDHF differs, with a greater role of NO in the basal tone and ACH-induced vasodilation at smaller vascular segments of ILA and AFF.

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Introduction

Endothelium, lining inside the vascular wall, participates importantly in the control of vascular tone. Accumulating evidence has accrued that endothelium produces a variety of vasoactive substances, including nitric oxide (NO), prostaglandins, and endothelium-derived hyperpolarizing factors (EDHF) [1–3]. These substances are

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believed to serve to modify the vascular tone. Thus, in the kidney the inhibition of NO synthesis causes prominent constriction of renal arterioles [4, 5], and enhances the vasoconstrictor response to angiotensin II [4] and endothelium [6]. Although these observations clearly indicate an important role of NO in the regulation of renal arteriolar tone, it has also been suggested that EDHF plays a substantial role in the renal arteriolar tone [7–9]. Thus, we have recently demonstrated that acetylcholine (ACH)-induced vasodilation of renal afferent arterioles is attributed to both NO-dependent and EDHF-dependent components [8, 9]; NO is responsible for the sustained phase, whereas EDHF plays a central role in the initial phase of the renal vasodilation.

The renal vasculature possesses marked heterogeneity in its functional as well as anatomical characteristics. The interlobular artery (ILA) originates from the arcuate artery, and tapers its diameter as it runs downstream to the surface of superficial cortex. In parallel with this change, the myogenic response of ILA differs, depending on the vessel diameter [5, 10]. Thus, greater vasoconstrictor responses to pressure are observed at the segment near the superficial cortex. In contrast, ILA segments near the arcuate artery manifest a diminished constriction. Recently, Takenaka et al. [10] have demonstrated that in large-caliber ILA segments, the myogenic vasoconstriction is mediated by PLC, whereas this constrictor response involves mechanosensitive cation channels in small-caliber ILA. These findings indicate distinct mechanisms for myogenic vasoconstrictor responses of ILA at the level of vascular smooth muscles. With regard to endothelial function, heterogeneity in the production of endothelial vasodilators has been reported in various vascular beds [11, 12]. Thus, Urakami-Harasawa et al. [11] have recently demonstrated that both NO and EDHF plays an important role in mediating the relaxation of large segments of gastroepiploic arteries, whereas EDHF is more important in the distal microvessels. These observations suggest that the role of NO and EDHF varies depending on the size of the coronary circulation. Nevertheless, this formulation has not been tested in the renal vasculature.

In the present study, we examined the vasodilator action of ACH on norepinephrine-induced constriction of afferent arterioles and ILA, using the isolated perfused hydronephrotic rat kidney [5, 8–10, 13, 14]. Furthermore, the role of NO and EDHF in modulating the vascular tone of afferent arterioles and ILA was evaluated in the presence of a NO synthase inhibitor, nitro-*L*-arginine methyl-ester (LNAME) and EDHF blockers, apamin and charyb-

dotoxin (CTx) [15, 16]. Finally, whether the segmental contribution of NO differs, depending on the size of the vessels, was examined.

Methods

All procedures involving this study were conducted following the guidelines of the Animal Care Committee of Keio University. The animals had free access to water and chows throughout the study. Chronic hydronephrosis was established to facilitate subsequent visualization of the renal microcirculation [5, 8–10, 13, 14]. Six-week-old male Wistar rats were anesthetized with ether. The right ureter was ligated through a small mid-abdominal incision. After 8–10 weeks, at which time renal tubular atrophy had progressed to a stage that allowed direct microscopic visualization of renal microvessels, the kidneys were harvested for perfusion study.

The rats were anesthetized with ether, and the abdominal cavity was exposed by midline incision. The renal artery of the hydronephrotic kidney was cannulated in situ through the superior mesenteric artery across the aorta. The hydronephrotic kidney was placed on the stage of an inverted microscope (IMT-2, Olympus, Tokyo, Japan) modified to accommodate a heated chamber equipped with a thin glass viewing port on the bottom surface. Kidneys were allowed to equilibrate for 30 min before initiating the experimental procedure.

Kidneys were perfused with medium consisting of a Krebs-Ringer bicarbonate buffer containing 5 mmol/l *D*-glucose, 7.5% bovine serum albumin (Sigma, St. Louis, Mo., USA), and a complement of amino acids [17]. The perfusion apparatus is illustrated in our previous publication [13]. The perfusion medium was saturated with a gas mixture of 95% O₂/5% CO₂ within a pressurized reservoir. Renal perfusate flow (RPF) was monitored by means of an extracorporeal electromagnetic flow probe (Model FF-015T, Nihon Kohden, Tokyo) placed in the perfusion circuit immediately proximal to the kidney. The perfusion pressure, monitored at the level of the renal artery, was maintained constant at 80 mm Hg by adjusting the back-pressure-type regulator (Model 10BP, Fairchild Industrial Products Co., Winston-Salem, N.C., USA).

Vessel diameters were measured as detailed previously [5, 8–10, 13, 14]. In brief, video images from a video camera (model XC-77, Sony, Tokyo) were recorded with a videocassette recorder and transmitted to a computer (PS55/Model 5551, IBM Japan, Tokyo) equipped with a video acquisition board (Targa 16+, Truevision Inc., Indianapolis, Ind., USA). Segments of ILA and afferent arterioles approximately 50 μ m in length were evaluated at 0.5- to 1-second intervals. Mean vessel diameter was determined by averaging all measurements obtained during the plateau of the response.

Experimental Protocols

Effect of ACH on NE-Constricted Afferent Arterioles and ILA. The effect of ACH on norepinephrine (NE; Sigma)-induced vasoconstriction of ILA and afferent arterioles was assessed. Initially, NE (0.3 μ mol/l) was administered to set basal vascular tone. Thereafter, ACH (Sigma) was added at concentrations of 0.01, 0.1 and 1 μ mol/l, and the vasodilator effect of ACH was evaluated. To eliminate pressure-induced changes in vessel diameter, renal perfusion pressure was maintained constant at 80 mm Hg throughout the study.

Role of NO and EDHF in ACH-Induced Renal Vasodilation. The role of NO and EDHF in mediating the ACH-induced vasodilation

of ILA and afferent arterioles was assessed during NE-induced vasoconstriction. Initially, LNAME (100 $\mu\text{mol/l}$; Sigma) or CTx (30 nmol/l; Sigma) + apamin (300 nmol/l; Sigma) was added to the renal perfusate. Thereafter, NE, at a final concentration of 0.3 $\mu\text{mol/l}$, was added to the perfusate. Following the observation of baseline vasoconstrictor responses, renal vasodilator actions of ACH (0.01, 0.1, and 1 $\mu\text{mol/l}$) were assessed. Since we previously observed that ACH-induced vasodilator responses comprised two components, i.e. initial (transient) and sustained phases, the vasodilator responses to ACH were assessed at 2–5 and 10 min, respectively.

Analysis of Data

Data are expressed as the means $\pm$ SEM. Data were 2-way analyzed by analysis of variance followed by Newman-Keul's multiple comparison post hoc test. $p < 0.05$ was considered statistically significant.

Results

Effect of ACH on NE-Constricted Afferent Arterioles and ILA

Figure 1 depicts the tracings illustrating the effect of ACH on NE-induced changes in RPF. As shown in the top panel, ACH elicited dose-dependent increases in RPF. Of note, the increase in RPF was sustained, although slight decrease was observed after initial responses. At 1 $\mu\text{mol/l}$, ACH elicited complete recovery of RPF (baseline, 12.7 ± 1.8 ; NE, 4.3 ± 0.6 ; ACH 10.3 ± 2.8 ml/min, $n = 5$). In the presence of LNAME (center); however, ACH caused transient increases in RPF. Thus, after initial increases, RPF was gradually decreased, returning to the pre-ACH level 10 min after ACH administration; at 1 $\mu\text{mol/l}$, the ACH-induced increase in RPF (from 3.5 ± 0.7 to 8.7 ± 1.6 ml/min, $n = 4$) abated to 5.8 ± 1.2 ml/min. In the presence of CTx and apamin (bottom), by contrast, the initial vasodilator response to ACH was blunted without affecting the sustained increase in RPF (initial, 11.7 ± 1.6 ; sustained, 11.4 ± 1.4 ml/min, $n = 5$).

The effects of ACH on NE-induced constriction of renal microvessels are summarized in figure 2. NE caused marked constriction of ILA (from 45.2 ± 4.4 to 29.0 ± 3.5 μm , $p < 0.01$, $n = 21$) and afferent arterioles (from 14.5 ± 0.6 to 9.6 ± 0.7 μm , $p < 0.01$, $n = 14$). The addition of 0.01 $\mu\text{mol/l}$ ACH caused sustained reversal of ILA constriction by $36 \pm 10\%$ ($p < 0.05$ vs. NE). Further administration of ACH elicited dose-dependent vasodilation, and produced $90 \pm 9\%$ reversal of NE-induced constriction of this vessel at 1 $\mu\text{mol/l}$ ($p < 0.01$). Similarly, ACH produced dose-dependent dilation of afferent arterioles (0.01 $\mu\text{mol/l}$, $39 \pm 5\%$; 0.1 $\mu\text{mol/l}$, $81 \pm 9\%$; 1 $\mu\text{mol/l}$, $117 \pm 13\%$; $n = 14$). The vasodilator responses of ILA did

Fig. 1. Effects of acetylcholine (ACH) on norepinephrine (NE)-induced changes in renal perfusate flow (RPF). ACH elicited sustained increases in PPF in a dose-dependent manner (a). In the presence of nitro-*L*-arginine methylester (LNAME), ACH-induced increases in RPF were converted to transient nature (b). The combined treatment with charybdotoxin (CTx) and apamin (K_{Ca} blockers), by contrast, impaired the initial phases of ACH-induced responses (c).

not differ from those of afferent arterioles ($p > 0.1$; fig. 2, bottom).

Role of NO and EDHF in ACH-Induced Renal Vasodilation

Figure 3 showed the effect of LNAME on ACH-induced vasodilation of renal microvessels. The addition of LNAME elicited a slight decrease in diameters of ILA (from 44.6 ± 3.6 to 41.6 ± 2.4 μm , $p < 0.01$, $n = 15$) and

Fig. 2. Effect of acetylcholine (ACH) on norepinephrine-induced vasodilation of renal microvessels. ACH elicited dose-dependent vasodilation of interlobular arteries and afferent arterioles (upper). When the above results are expressed as percent inhibition of norepinephrine-induced constriction, the responses of interlobular arteries (squares) and afferent arterioles (circles) exhibit similar vasodilator responses (lower). Results are mean $\pm$ SEM. * $p < 0.05$ vs. norepinephrine.

Fig. 3. Effect of nitric oxide inhibition on initial and sustained phases of acetylcholine (ACH)-induced vasodilation of renal microvasculature. The administration of nitro-*L*-arginine methylester (LNAME) inhibited the sustained phase of ACH-induced dilation in both interlobular arteries (ILA) and afferent arterioles (filled bars). In contrast, LNAME had no effect on the initial phase of ACH-induced dilation. Open bars indicate the responses in the absence of LNAME. Results are mean $\pm$ SEM. * $p < 0.05$; ** $p < 0.01$.

Fig. 4. Effect of potassium channel inhibition on initial and sustained phases of acetylcholine (ACH)-induced vasodilation of renal microvasculature. The administration of charybdotoxin and apamin inhibited the initial phase of ACH-induced dilation in both interlobular arteries (ILA) and afferent arterioles (filled bars). In contrast, the treatment with these agents did not alter the sustained phase of ACH-induced dilation. Open bars indicate the responses in the absence of charybdotoxin and apamin. Results are mean $\pm$ SEM. * $p < 0.05$; ** $p < 0.01$.

afferent arterioles (from 14.4 ± 0.7 to 12.6 ± 0.6 μm , $p < 0.01$, $n = 9$). In the presence of LNAME, NE produced marked constriction of ILA (26.0 ± 2.3 μm , $p < 0.01$) and afferent arterioles (8.0 ± 0.7 μm , $p < 0.01$). In this setting, ACH-induced vasodilation of ILA abated with time. Thus, the initial phase of the ILA response did not differ from that in the absence of LNAME. In contrast, after 10 min of ACH administration, the sustained phase of the ILA dilator response was markedly impaired, compared with that of control (0.1 $\mu\text{mol/l}$, $42 \pm 7\%$, $p < 0.01$ vs. $79 \pm 7\%$; 1 $\mu\text{mol/l}$, $53 \pm 7\%$, $p < 0.01$ vs. $97 \pm 8\%$). Similarly, LNAME blunted the sustained response of afferent arterioles (0.01 $\mu\text{mol/l}$, $4 \pm 7\%$, $p < 0.05$ vs. $39 \pm 5\%$; 0.1 $\mu\text{mol/l}$, $19 \pm 6\%$, $p < 0.01$ vs. $81 \pm 9\%$; 1 $\mu\text{mol/l}$, $32 \pm 7\%$, $p < 0.01$ vs. $117 \pm 13\%$), without affecting the initial vasodilation. When compared with the ILA response to ACH, afferent arteriolar vasodilation was diminished at 0.1 $\mu\text{mol/l}$ ($p < 0.01$) and 1 $\mu\text{mol/l}$ ($p < 0.05$).

The Ca-activated potassium channel (K_{Ca}) inhibition by combined treatment with CTx and apamin did not alter the basal diameter of ILA ($p > 0.5$) or afferent arterioles ($p > 0.1$). The treatment with these agents exerted the inhibitory action on initial vasodilator responses to ACH (fig. 4). Thus, at 0.1 $\mu\text{mol/l}$, ACH-induced ILA dilation was markedly diminished ($38 \pm 9\%$, $n = 11$, $p < 0.01$ vs.

$88 \pm 12\%$). The ILA response to 1 $\mu\text{mol/l}$ ACH ($46 \pm 9\%$, $n = 11$) was also less than that seen in control ($p < 0.01$). Similarly, CTx + apamin blunted the initial phases of afferent arteriolar response to 0.1 ($42 \pm 13\%$, $n = 9$, $p < 0.05$) and 1 $\mu\text{mol/l}$ ACH ($58 \pm 8\%$, $n = 9$, $p < 0.01$). In contrast, these combined treatments had no effect on the sustained phase of the ACH-induced vasodilation of ILA or afferent arterioles.

Segmental Role of NO and EDHF in Vascular Tone

Since the diameter of renal microvessels examined varied widely (e.g. from 10 to 100 μm), whether the role of NO and EDHF differed depending on the basal diameter was examined. Figure 5 illustrates the relationship between basal vessel diameters and the responses to LNAME (top) or NE (bottom). LNAME-induced vasoconstriction of ILA with diameter less than 30 μm ($10 \pm 2\%$, $n = 6$) was nearly the same as that of afferent arterioles ($9 \pm 3\%$, $n = 14$). ILA with greater diameters, however, manifested a blunted constrictor response to LNAME (30–60 μm , $6 \pm 3\%$, $n = 13$, $p < 0.05$; >60 μm , $3 \pm 3\%$, $n = 8$, $p < 0.01$). In contrast, NE-induced constriction of renal microvessels was not associated with basal vessel diameter.

The reversal by ACH of NE-induced renal microvascular constriction was estimated in the presence of LNAME

Fig. 5. Diameter-dependency of nitro-*L*-arginine methylester (LNAME)-induced constriction of renal microvessels. Results are mean $\pm$ SEM. AFF = Afferent arterioles. * $p < 0.05$, ** $p < 0.01$ vs. AFF.

and CTx + apamin, and the responses were categorized based on the basal vessel diameter (fig. 6). In the presence of LNAME, the sustained vasodilator response to ACH was impaired at smaller vessel segments. In contrast, the initial responses to ACH in the presence CTx + apamin were not affected by the vessel diameter ($p > 0.2$).

Discussion

Recent studies have demonstrated that endothelium constitutes an important structure, participating in the modulation of vascular tone by producing a variety of vasoactive substances [1–3]. NO is constitutively produced within the kidney, and plays a pivotal role in mediating the vascular tone [4, 5, 18]. Furthermore, EDHF, acting presumably via K_{Ca} channels [14, 19, 20], contributes to the regulation of vascular tone. Urakami-Harasawa et al. [11] have demonstrated EDHF constitutes a major vasodilator substance regulating the vascular tone of human gastroepiploic arteries, and its contribution to the vascular tone is greater in microvessels than in large arteries. In the renal vasculature, substantial evidence for the activity of EDHF has been provided [8, 9, 21]. We have recently demonstrated that both NO and

EDHF contribute to the vasodilator action of ACH on afferent arterioles [8, 9]. Nevertheless, whether the relative role of NO and EDHF in the control of renal vascular tone differs has not been delineated in ILA and afferent arterioles.

In the present study, we have demonstrated that ACH produces prominent vasodilation of ILA and afferent arterioles; these vasodilator responses were nearly the same in ILA and afferent arterioles (fig. 2). Furthermore, in the presence of LNAME, the sustained vasodilator response to ACH was converted to transient dilation (fig. 1, 3) in both microvessels. These observations suggest that NO contributes to the sustained vasodilator action of ACH in these vessels, and is responsible for the majority of the ACH-induced vasodilation. Alternatively, the initial transient vasodilation of these vessels is not attributed to NO, but other factors contribute to this dilation. Indeed, the initial peak response was blunted by the combined treatment with CTx and apamin, K_{Ca} channel blockers (fig. 1, 4), a finding suggestive of the involvement of membrane hyperpolarization [22]. In this regard, previous studies demonstrated that the transient component of ACH-induced vasodilation of mesenteric arteries paralleled membrane hyperpolarization, but not NO production [23]. In contrast, ACH failed to inhibit KCl-induced membrane depolarization or vascular contraction [8, 23]. In concert, it is most likely that the initial vasodilator action of ACH in both afferent arterioles and ILA is mediated by EDHF.

Although renal microvessels manifest wide anatomical and functional heterogeneity, it has not been examined whether the role of NO and EDHF differs along the vessel segments. It has been reported that the characteristics of preglomerular renal microcirculation differs along the length of the vessels. We previously demonstrated that myogenic reactivity of the ILA to pressure varied, with greater responses occurring in the more distal segments [5]. Takenaka et al. [10] also indicated that mechanisms mediating myogenic responses differ among distinct segments of ILAs. Such segmental heterogeneity at the vascular smooth muscle cell would allow speculation that endothelial function may also vary along the length of the vessel. Thus, the present study demonstrates that under basal vascular tone, LNAME constricts renal preglomerular vessels, with greater action on the more distal (i.e. small-caliber) arterioles (fig. 5). Furthermore, during the NO inhibition by LNAME, the ACH-induced vasodilation is more pronouncedly impaired in the distal segments of renal microvessels, compared with the more proximal portion of these vessels (fig. 6). These observations strong-

Fig. 6. Relationship between the baseline vessel diameter and the acetylcholine (ACH)-induced changes in diameter in the presence of nitro-*L*-arginine methylester (LNAME) and charybdotoxin + apamin. In the presence of LNAME (filled bars), ACH-induced sustained vasodilation of norepinephrine-constricted vessels was diminished as the baseline vessel diameter was smaller. In contrast, the inhibition of calcium-activated potassium channels (K_{Ca}) by charybdotoxin + apamin prevented the initial phase of ACH-induced vasodilation in all sizes of renal microvessels examined (hatched bars). Open bars indicate the responses in the absence of LNAME or charybdotoxin + apamin. Results are mean $\pm$ SEM. AFF = Afferent arterioles; NO = nitric oxide. * $p < 0.05$; ** $p < 0.01$. † $p < 0.05$ and †† $p < 0.01$ vs. AFF.

ly suggest that NO participates in the regulation of basal vascular tone as well as ACH-induced dilation, with greater contribution in smaller vessels. In this regard, in coronary circulation, NO is reported to constitute a major factor mediating the ACH-induced vasodilation of large ($>100 \mu\text{m}$ in diameter) coronary arteries, whereas the relative role of NO in the ACH-induced vasodilation is diminished, with EDHF participating in its action in small ($<100 \mu\text{m}$) arterioles [12]. Similar tendency is demonstrated in mesenteric arteries, with smaller contribution of NO in the microvessels ($100\text{--}150 \mu\text{m}$) than in large arteries [11, 24]. Of note, the vessel diameters observed in their studies are larger than those seen in the present study, and the ILA diameter ($20\text{--}100 \mu\text{m}$) corresponds to the small vessels in their reports. Irrespective of these dif-

ferences, these observations are consistent with the formulation that the role of NO in both basal and ACH-stimulated vascular tone varies depending on the size of renal vascular beds.

In contrast to the renal vascular action of NO, the combined treatment with CTx and apamin had no effect on basal diameters of ILA or afferent arterioles. Furthermore, the initial vasodilator responses to ACH were nearly the same in ILA and afferent arterioles, without dependency on basal vessel diameter (fig. 6). It has been reported that EDHF mediates the ACH-induced vasodilation in a variety of vascular beds, including coronary arteries [12], mesenteric arteries [11, 24], hepatic arteries [25] and aorta [6], and the activity differs depending on the size of vascular beds [11, 24]. In the present study,

however, we found that the EDHF-mediated vasodilation of renal microvessels was only transient when induced by ACH, and it does not appear to contribute to the sustained effect of ACH on renal arteriolar tone in our experimental condition. In contrast, previous studies demonstrated sustained [11, 26], as well as transient [8, 9, 23], vasodilator action of EDHF. Furthermore, EDHF-mediated vasodilation is shown to be vessel size-dependent (see above). Interestingly, there have been identified multiple EDHFs, including epoxyeicosatrienoic acids [21, 27] and K ion per se [28], which affect the activity of potassium channels. In concert, these observations suggest heterogeneity in EDHFs in mediating endothelium-dependent vasodilation.

Of note, in large proximal segments of ILA, the inhibition of NO only slightly impaired the ACH-induced vasodilation (fig. 6). In consideration of transient vasodilator activity of EDHF in our experimental conditions, it is reasonable to speculate that additional factors also participate in the sustained components of ACH-induced vasodilation of renal microvessels. Indeed, our previous studies demonstrated that prostaglandins contribute in part to the ACH-induced sustained dilation of afferent arterioles [8]. Moreover, in our preliminary study, the combined

pretreatment with LNAME and indomethacin (100 $\mu\text{mol/l}$) caused further reductions in the sustained component of ACH-induced ILA dilation (0.01 $\mu\text{mol/l}$, $12 \pm 9\%$ reversal; 0.1 $\mu\text{mol/l}$, $16 \pm 8\%$ reversal; 1 $\mu\text{mol/l}$, $21 \pm 10\%$ reversal) [unpubl. observation]. In concert, prostaglandins may participate in the acetylcholine-induced vasodilation, particularly at the large-caliber renal microvascular segments. Alternatively, the inhibition of NO might stimulate vasoconstrictor eicosanoids (e.g. 20-HETE) [29], which could modify the vascular tone of ILA.

In summary, the present study demonstrates that the renal microvessels produce at least two potent vasoactive substances, NO and EDHF; the former contributes to the sustained vasodilation, while the latter participates in the initial (transient) phase of ACH-induced vasodilation. Furthermore, NO constitutes an important determinant of renal microvascular tone under basal conditions as well as ACH stimulation, with greater role of NO at smaller caliber-size arterioles. In contrast, EDHF exerts its vasodilator action, similar in magnitude in ILA and afferent arterioles. Thus, the relative contribution of NO and EDHF to the arteriolar tone varies, depending on the localization of the vascular segment.

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