Multiple Factors Contribute to Acetylcholine-Induced Renal Afferent Arteriolar Vasodilation During Myogenic and Norepinephrine- and KCl-Induced Vasoconstriction

Studies in the Isolated Perfused Hydronephrotic Kidney

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Abstract Acetylcholine (ACh) elicits vasodilation by releasing a number of endothelium-derived relaxing factors (EDRFs). We used the isolated perfused hydronephrotic rat kidney to examine the characteristics of ACh-induced vasodilation of renal afferent arterioles during different types of underlying vasoconstriction. Basal arteriolar tone was increased by either elevating perfusion pressure to 180 mm Hg (myogenic), administering 0.3 \mu mol/L norepinephrine (NE), or elevating medium potassium concentration to 30 mmol/L (KCl). ACh (10 \mu mol/L) completely reversed myogenic and NE-induced vasoconstriction and reversed KCl-induced vasoconstriction by 80±5%. However, whereas ACh produced a sustained vasodilation during KCl- and NE-induced vasoconstriction, only a transient reversal of myogenic vasoconstriction was observed, and myogenic tone recovered within 5 to 10 minutes. ACh-induced vasodilation of arterioles preconstricted with KCl was markedly inhibited by either indomethacin (100 \mu mol/L) or nitro-L-arginine (100 \mu mol/L) and was completely abolished by pretreatment with both inhibitors. In contrast, indomethacin and nitro-L-arginine had no effect on the transient response to ACh observed during pressure-induced vasoconstriction. In vessels preconstricted with NE, nitro-L-arginine converted the normally sustained response to ACh to a transient vasodilation, which was refractory to both nitric oxide synthase and cyclooxygenase inhibition. Since this component was not observed during KCl-induced vasoconstriction, it may reflect the actions of an, as yet unidentified, endothelium-derived hyperpolarizing factor (EDHF). Our findings thus suggest that prostanooids, nitric oxide, and EDHF all contribute to ACh-induced renal afferent arteriolar vasodilation and that the relative contributions of these individual EDRFs depends on the nature of the underlying renal vascular tone. (Circ Res. 1994;75:821-828.)

Keywords • acetylcholine • nitric oxide • prostaglandins • endothelium-derived hyperpolarizing factor • renal microcirculation

Acetylcholine (ACh) induces vasodilation by stimulating the release of endothelium-derived relaxing factors (EDRFs) in a variety of vascular beds.1-3 Although nitric oxide (NO) appears to account for the actions of ACh in many settings,4 elimination of NO (eg, by hemoglobin or NO-synthase inhibitors) does not always prevent endothelium-dependent vasodilation.5-7 Thus, evidence has accrued suggesting that EDRFs other than NO may contribute to vascular actions of ACh.3,8-11 It is well appreciated that ACh stimulates the endothelial production of vasodilatatory prostaglandins.12-14 Furthermore, recent evidence suggests that an endothelium-derived hyperpolarizing factor (EDHF) may also contribute to the vasodilatory actions of ACh.5,10 Thus, EDRFs distinct from NO may mediate a part of the ACh-induced vasodilation. ACh is a potent vasodilator of the renal afferent arteriole15-19; however, the mechanisms mediating the renal microvascular responses to ACh are not clearly defined.

NO promotes vasodilation by stimulating soluble guanylate cyclase and increasing intracellular levels of cGMP.20,21 We have previously demonstrated that in isolated perfused hydronephrotic rat kidneys, myogenic or pressure-induced vasoconstriction of the afferent arteriole is insensitive to the vasorelaxant action of cGMP-related substances, including atrial natriuretic peptide (ANP), nitroprusside, and 8-bromo-cGMP.22 These findings suggested a reasonable approach in the investigation of the possible role of non-NO EDRFs in the renal microvascular response to ACh. Since NO acts via cGMP and cGMP has little effect on myogenic vasoconstriction in our model, we reasoned that investigations with this model may reveal a contributory role of EDRFs other than NO in the renal microvasculature. Thus, during pressure-induced afferent arteriolar vasoconstriction, EDHF and prostaglandins may play a greater role than NO in ACh-induced vasodilation. On the other hand, when the same vessel is constricted by elevating external potassium,23 the anticipated effects of a hyperpolarizing factor would be minimal.9-11 Therefore, we investigated the effects of ACh on myogenic and norepinephrine- and KCl-induced vasoconstriction of the afferent arteriole in the isolated
perfused hydropnephrotic rat kidney. We examined the effects of nitro-L-arginine (N-Arg) and indomethacin on ACh-induced vasodilation to determine the relative contributions of NO and prostaglandins to ACh-induced vasoconstriction in each setting.

Materials and Methods

Animal Preparation

Chronic hydropnephrosis was established to facilitate direct visualization of the renal microcirculation. Six-week-old male Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, Mass) were anesthetized with methoxyflurane (Pitman-Moore). The right ureter was ligated through a small abdominal incision. After 8 to 10 weeks, tubular atrophy of the right kidney had progressed to a stage that allowed direct microscopic visualization of the renal microvessels. The hydropnephrotic kidneys were excised and perfused in vitro as described below and detailed in previous communications.

Perfusion of Hydropnephrotic Kidneys

The animals were anesthetized with methoxyflurane. The renal artery of the hydropnephrotic kidney was cannulated in situ by introducing the arterial cannula through the superior mesenteric artery and across the aorta. An infusion of warm oxygenated medium was administered during the cannulation procedure and was continued during isolation and excision of the kidney. The hydropnephrotic kidney was perfused on an inverted microscope (Nikon, model K) modified to accommodate a heated chamber equipped with a thin-glass viewing port on the bottom surface. Kidneys were allowed to equilibrate for at least 30 minutes before initiating the experimental protocol.

The apparatus used to study the hydropnephrotic kidney in vitro is illustrated in previous publications. The perfusate consisted of a Krebs-Ringer bicarbonate buffer containing (mmol/L) sodium 145, potassium 5, MgSO4 2.4, CaCl2 2.0, and glucose 5, along with 7.5% bovine serum albumin (Bovuminar, Armour Pharmaceutical Co) and a complement of amino acids as detailed previously. The perfusate was saturated with 95% O2/5% CO2. Perfusion pressure was monitored within the renal artery and was altered by adjusting a back-pressure-type regulator (model 10BP, Fairchild Industrial Products Co), controlling the exit of gas from the pressurized reservoir.

Vessel diameters were measured as detailed previously. Video images were recorded, transmitted to an IBM-AT computer equipped with a video acquisition and display board (model IVG-128, Datacube), and analyzed by custom-designed image processing. Vessel segments ~50 μm in length were analyzed at 2- to 5-second intervals. Diameters were estimated from the mean distance between parallel edges of the selected vascular segment. Mean vessel diameters were obtained by averaging all determinations obtained during the plateau of the response.

Experimental Protocols

The vasodilatory effects of ACh (Sigma Chemical Co) were assessed under three experimental conditions: during KCl-induced, norepinephrine (NE)-induced, and pressure-induced vasoconstriction. In the first series of experiments, after the determination of basal afferent arteriolar diameters, the potassium concentration of the perfusate was elevated from 5 to 30 mmol/L by the isosmotic addition of medium in which KCl was substituted for NaCl. ACh was then added directly into the perfusate to achieve the concentrations desired.

In a second series of experiments, the effects of ACh on the NE-induced vasoconstriction were assessed. In these studies, 0.3 mmol/L NE was administered to elevate afferent arteriolar tone, and the vasodilatory response to ACh was then examined. To eliminate a possible contribution of pressure-induced changes in vascular tone during treatment with KCl or NE, renal arterial pressure (RAP) was maintained constant at 80 mm Hg during the entire experimental procedure when these agents were administered.

We have previously demonstrated that an increase in RAP elicits marked afferent arteriolar vasoconstriction (ie, myogenic vasoconstriction) in the isolated perfused hydropnephrotic kidney. In the third series of experiments, we examined the effects of ACh on the pressure-induced vasoconstriction of this vessel. Initially, RAP was maintained constant at 80 mm Hg. Thereafter, RAP was elevated to 180 mm Hg to elicit myogenic vasoconstriction. ACh at final concentrations ranging from 10 nmol/L to 10 μmol/L was then administered into the medium, and the afferent arteriolar responses were observed continuously at least for 20 minutes.

N-Arg (Sigma) and indomethacin (Sigma) were used to assess the contributions of NO and prostaglandins to ACh-induced vasodilation. Either N-Arg (100 μmol/L) or indomethacin (100 μmol/L) was added to the perfusate before induction of the afferent arteriolar vasoconstriction by KCl, NE, or pressure. The afferent arteriolar response to ACh was then assessed in the same settings as described above. In those studies in which N-Arg was administered, L-arginine was omitted from the perfusate.

Analysis of Data

All data are expressed as mean±SEM. Data were analyzed by one-way ANOVA followed by Student's t test. Changes within experimental groups were subjected to paired analysis; differences between groups were assessed by unpaired analysis. Values of P<.05 were considered statistically significant. For multiple comparisons, values of P<.05/n (where n indicates the number of comparisons) were considered significant.

Results

Effects of ACh on KCl-Induced Vasoconstriction

The representative tracing in Fig 1 illustrates the effects of ACh on KCl-induced vasoconstriction in a renal afferent arteriole. KCl (30 mmol/L) decreased the arteriolar diameter from 20.3 to 13.1 μm. ACh (10 μmol/L) elicited a sustained, albeit incomplete, vasodilation and increased the diameter to 18.0 μm. The subsequent administration of 1 μmol/L nifedipine completely reversed the remaining vasoconstriction, increasing the diameter to 19.8 μm. In five afferent arterioles treated similarly, KCl decreased the afferent arteriolar diameter from 19.2±0.4 to 12.5±0.2 μm (P<.001). Administration of 10 μmol/L ACh elicited a sustained vasodilation (ie, diameters at 5, 10, and 15 minutes: 17.1±0.4, 17.2±0.4, and 17.4±0.4 μm, respec-
Fig 2. Graph showing the effects of 100 µmol/L indomethacin (Indo) and 100 µmol/L nitro-L-arginine (N-Arg) on acetylcholine-induced vasodilation of arterioles preconstricted with KCl. Note that both indomethacin and N-Arg partially abolished acetylcholine-induced vasodilation. Values are mean±SEM (n=12 for control, n=6 for Indo, n=5 for N-Arg, and n=5 for Indo+N-Arg).

Fig 3. Original tracings illustrating the nature of acetylcholine (ACh)-induced vasodilation in arterioles preconstricted with noradrenaline during control conditions (top tracing), after treatment with 100 µmol/L nitro-L-arginine (N-Arg, center tracing), or after combined treatment with 100 µmol/L N-Arg and 100 µmol/L indomethacin (N-Arg+Indo, lower tracing).
Fig 4. Bar graph showing effects of nitro-l-arginine (N-Arg, 100 μmol/L) and N-Arg plus 100 μmol/L indomethacin (Indo) on acetylcholine-induced vasodilation of arterioles preconstricted with 0.3 μmol/L norepinephrine (NE). N-Arg and Indo had no effect on the initial vasodilation (ie, 1- to 2-minute duration) but abolished the sustained component of the response (ie, 10 minutes).

no further effect on the transient vasodilatory response (112±2% reversal at 1 to 2 minutes) but eliminated all vestiges of the sustained phase of vasodilation (2±1% reversal after 10 minutes, n=5).

Effects of ACh on Pressure-Induced Vasoconstriction

The tracing depicted in Fig 5 illustrates ACh-induced vasodilation of an afferent arteriole preconstricted by elevated pressure. Elevating renal arterial pressure from 80 to 180 mm Hg reduced afferent arteriolar diameter from 23.4 to 17.5 μm. The administration of 10 μmol/L ACh resulted in a marked vasodilation, during which diameter reached a peak value (25.3 μm) within 2 minutes. After this peak vasodilation, arteriolar diameter spontaneously returned toward pre-ACh levels. Within 7 minutes, afferent arteriolar diameter decreased to 18.7 μmol/L, even in the continued presence of ACh. On subsequent reduction of RAP from 180 to 80 mm Hg, arteriolar diameter returned to its original value (23.8 μm). Mean data obtained using this protocol are summarized in Fig 6. Increasing RAP to 180 mm Hg reduced afferent arteriolar diameter by 23.4±2.5% (ie, from 21.0±1.1 to 16.1±0.8 μm; P<.005). ACh administration (10 μmol/L) caused a complete but transitory inhibition of myogenic tone, increasing mean diameter to 22.7±1.3 μm within 2 minutes. During the peak response, vessel diameters increased to values greater than that seen in the control condition (108.0±0.9%), reflecting a passive, pressure-induced distention of the vessel in the absence of myogenic tone. After 10 minutes in the continued presence of 10 μmol/L ACh, arteriolar diameters had returned to 170±0.8 μm (−18.9±2.2% change from 80 mm Hg, P<.001), and only a modest albeit significant vasodilatory response to ACh remained (P<.005 compared with pre-ACh diameters). On returning RAP to 80 mm Hg, arteriolar diameters returned to their initial values (21.2±1.1 μm, P>.5).

Thus, during pressure-induced vasoconstriction, ACh elicited a transitory vasodilation. This characteristic response was similar to that observed during NE-induced vasoconstriction in the presence of N-Arg (see above). As illustrated in Fig 7, N-Arg and indomethacin did not alter the transient ACh-induced vasodilation of vessels preconstricted with elevated pressure, nor did they alter the vasoconstrictor response to pressure. Increasing RAP from 80 to 180 mm Hg reduced afferent arteriolar diameter by 23.4±2.5% (n=5) in control vessels and by 22.9±2.2% (n=5) and 24.0±3.0% (n=6) in vessels treated with N-Arg and indomethacin, respectively (P>.5 versus control vessels). In control vessels, ACh increased diameters to 136.8±6.8% of basal diameters within the first 2 minutes (n=5). After pretreatment with either N-Arg or indomethacin, ACh increased diameters to 141.3±17.2% (n=5) and 172.9±13.8% (n=6) of basal diameters, respectively. In each case, the calculated percent inhibition was >100%, indicating an absence of myogenic tone and pressure-induced arteriolar distention. As seen in the control vessels, the ACh-induced vasodilation abated with time. After 10 minutes of exposure to ACh, the level of inhibition remaining in kidneys treated with N-Arg was 22.7±2.8%, a value not different from that observed with control kidneys (19.8±1.8%, P>.05). However, this residual vasodilation was abolished in kidneys treated with indomethacin.
(5.2±5.6% inhibition, P<.025). Thus, in the indomethacin-treated group, afferent arteriolar diameters returned to pre-ACh values (14.5±0.8 μm at 10 minutes versus pre-ACh value of 14.8±0.7 μm, P>.1).

Finally, we examined the effects of combined treatment with N-Arg (100 μmol/L) and indomethacin (100 μmol/L) on the early phase (ie, peak) of ACh-induced vasodilation. In the absence of these inhibitors, 10 nmol/L, 0.1 μmol/L, 1.0 μmol/L, and 10 μmol/L ACh inhibited myogenic tone by 15.4±4.3%, 53.0±6.4%, 111.0±8.4%, and 144.5±10.2%, respectively (open circles, Fig 8). After pretreatment with both 100 μmol/L N-Arg and 100 μmol/L indomethacin (closed circles, Fig 8, n=6), myogenic tone was inhibited identically: 18.1±2.6% (P>.5), 44.4±7.3% (P>.4), 107.3±10.8% (P>.5), and 126.4±12.1% (P>.2) at 10 nmol/L, 0.1 μmol/L, 1.0 μmol/L, and 10 μmol/L ACh, respectively. The IC50 for ACh-induced afferent arteriolar vasodilation in the absence of the inhibitors (89.6±12.4 nmol/L) was similar to that observed after combined pretreatment (124.4±34.1 nmol/L, P>.2). Thus, even when administered simultaneously, N-Arg and indomethacin had no effect on the transient ACh-induced vasodilation of vessels preconstricted with elevated pressure.

**Discussion**

The results of the present study underscore the complex nature of the renal microvascular response to ACh. It is well established that the vasodilatory actions of ACh are endothelium dependent and that ACh promotes vasodilation by stimulating the endothelial release of NO. Nevertheless, NO does not fully account for the endothelium-dependent actions of ACh.2,3,5,8,29-31 In the present study, we describe ACh-induced renal vasodilatory responses that were either dependent or independent of NO synthase and cyclooxygenase, depending on the intervention used to establish basal renal vascular tone. These findings demonstrate not only that EDRFs other than NO play a prominent role in ACh-induced vasodilation in the renal vasculature but also that the relative contributions of NO, prostanoids, and other EDRFs (ie, EDHF) as determinants of this response depend on the nature of the underlying renal vascular tone.

In the present study, ACh dilated afferent arterioles that were preconstricted with either KCl, NE, or elevated renal perfusion pressure. However, the characteristics of ACh-induced vasodilation differed substantially in each of these experimental settings. First, whereas ACh caused sustained vasodilation of arterioles constricted with KCl and NE, it produced only a transient vasodilation of arterioles preconstricted by elevated pressure. Second, at the highest concentration (10 μmol/L), ACh only partially reversed KCl-induced vasoconstriction (ie, 80±5% reversal) but completely reversed the vasoconstrictor responses to NE and pressure. Finally, blockade of NO and prostaglandin synthesis produced markedly different effects in each setting. During KCl-induced vasoconstriction, ACh-induced vasodilation was substantially inhibited by either N-Arg or indomethacin and was completely prevented by the combined treatment with these two agents. In contrast, the transient vasodilation observed during myogenic vasoconstriction was totally unaffected by N-Arg and/or indomethacin treatment. The pharmacological characteristics of ACh-induced vasodilation of vessels preconstricted with NE fell somewhat between these two extremes. N-Arg treatment fully inhibited the sustained component of ACh-induced vasodilation during NE-induced vasoconstriction. However, this treatment unmasked an initial transient dilation that was insensitive to N-Arg or indomethacin and thus similar in nature to the ACh-induced response observed in vessels preconstricted with elevated pressure. These observations indicate differing mechanisms of ACh-induced vasodilation in each of these three settings.

Previous in vivo studies have demonstrated an obligatory role of NO in the vasodilatory response of the renal microcirculation to ACh. Tolins et al32 and Lahera et al33 reported that ACh-induced renal vasodilation is largely prevented by N°-monomethyl-L-arginine. These effects were reversed by excess L-arginine, demonstrating a dependence on NO synthase. On the other hand, evidence also suggests that factors other than NO contribute to ACh-induced renal vasodilation. For example,
Majid and Navar13 demonstrated that in the presence of N-Arg, indomethacin attenuates ACh-induced increases in renal blood flow in vivo, indicating that vasodilatory prostanoids mediate a component of ACh-induced renal vasodilation. ACh stimulates endothelial production of both NO and vasodilatory prostaglandins.4,12

In the present study, indomethacin and N-Arg blocked ACh-induced vasodilation to similar degrees during KCl-induced vasoconstriction. In this setting, it appears that both NO and prostanoid production are required for the full vasodilatory response to ACh, suggesting synergistic actions of these two EDRFs. NO and prostanoids promote vasodilation by augmenting intracellular cGMP and cAMP, respectively. Studies in isolated aortic smooth muscle indicate that vasoconstriction elicited by KCl-induced depolarization is less sensitive to cGMP than is the vasoconstriction elicited by other means (eg, NE34). Similarly, studies with aortic tissues suggest that KCl-induced vasoconstriction is also relatively insensitive to the actions of cAMP.35 In the present study, we observed only a very modest degree of inhibition by ACh of KCl-induced vasoconstriction after treatment with either N-Arg (29.3 ± 4.1%) or indomethacin (39.2 ± 5.6%), whereas in the absence of these two blockers, ACh reversed KCl responses by 80.1 ± 4.9%. Furthermore, pretreatment with both blockers completely inhibited the arteriolar responses to ACh (5.6 ± 3.4%). Thus, during KCl-induced vasoconstriction, the combined effects of ACh-induced release of NO and prostanoids were much greater than the individual actions of these two EDRFs. Both factors were required to attain maximal vasodilation in this setting.

When the afferent arteriole was preconstricted by elevating perfusion pressure, a different type of ACh-induced vasodilation was observed. In this setting, ACh elicited a complete vasodilation that was transient, followed by a small residual vasodilatory response. The initial response was not affected by indomethacin and/or N-Arg, whereas the residual response was eliminated by indomethacin but not N-Arg. Previous studies from our laboratory have demonstrated that myogenic vasoconstriction of the renal afferent arteriole is very refractory to the actions of cGMP–dependent vasodilators.22 This is illustrated in Fig 9 (data obtained from Reference 22). Note that the NO donor sodium nitroprusside is far more effective in reversing afferent arteriolar vasoconstriction induced by NE than that induced by pressure. In a similar fashion, we have found myogenic tone to be insensitive to ANP and membrane-permeant cGMP analogues in this model.22 Accordingly, one would not anticipate ACh-induced NO release to influence myogenic responses in our preparation.

Although N-Arg did not alter ACh-induced vasodilation during pressure-induced vasoconstriction, indomethacin abolished the small albeit persistent vasodilation normally remaining after 10 minutes. We have previously demonstrated that prostaglandin E2 is capable of inhibiting myogenic vasoconstriction in this model.19 Thus, the present results suggest that prostaglandins may contribute to the small sustained component of ACh-induced vasodilation during pressure-induced vasoconstriction. However, neither prostaglandins nor NO mediate the initial transient vasodilation observed in this setting. As discussed below, this response may reflect the actions of the purported hyperpolarizing factor (EDHF).

The results of our studies examining the nature of ACh-induced vasodilation during NE-induced vasoconstriction also suggest contributions from multiple EDRFs. In this setting, ACh normally produced a sustained vasodilation. The sustained component of this response, however, was completely blocked by N-Arg. As discussed above, NE-induced vasoconstriction is quite sensitive to cGMP-dependent vasodilators such as NO (eg, Fig 9). Similarly, Ito and colleagues37,18 demonstrated that the afferent arteriolar responses to angiotensin II17 and endothelin18 are very sensitive to alterations in basal NO production. Such observations would explain the marked effects of NO synthase inhibition on renal hemodynamics and vasodilatory responses to ACh in experimental settings in which renin levels and renal sympathetic tone are enhanced. However, in our studies a transient ACh-induced dilation remained after NO synthase blockade and/or cyclooxygenase inhibition, suggesting the actions of a third EDRF. Similarly, Nagao and Vanhoutte11 have reported that the normally sustained ACh-induced vasodilation in rat femoral vein is converted to a transient response by N-Arg.

The transient vasodilatory component of the ACh-induced response in arterioles preconstricted by pressure or NE is mediated by a factor distinct from NO or prostaglandins. The temporal character of this response is similar to that ascribed to an EDHF.30,31 The ACh-induced vasodilations decayed with a time constant of \( \approx 2 \) minutes, completely subsiding within \( \approx 10 \) minutes. In rat aorta, main pulmonary artery,30,31 and femoral vein,11 the application of 10 \( \mu \)mol/L ACh results in a transient hyperpolarization that decays with a time constant of \( \approx 2 \) minutes31 and completely subsides within 10 minutes. This hyperpolarizing response is abolished on removal of the endothelium11,30,31 but is not affected by hemoglobin,30 methylene blue,30,31 N-Arg,11 or indomethacin.30 Furthermore, with one exception,36 reports indicate that ACh-induced hyper-
polarization is not mimicked by application of exogenous NO or nitroglycerin.\textsuperscript{10-12,37,38}

In many vascular preparations, ACh-induced hyperpolarization is dissociated from vasodilation.\textsuperscript{11,39} This is interpreted as suggesting that ACh releases both NO and EDHF but that the former plays a more prominent role in the vasodilatory response.\textsuperscript{11} If, as we suggest, pressure-induced vasoconstriction in our model is refractory to the actions of NO, EDHF may represent the major factor responsible for mediating ACh-induced vasodilation in this setting. In contrast, one would not anticipate EDHF to influence KCl-induced vasoconstriction. During KCl-induced depolarization, the membrane potential is essentially clamped to that of the potassium equilibrium potential (eg, \(-40 \text{ mV}\)) and would not be affected by most hyperpolarizing stimuli. For example, in the rat femoral vein, ACh elicits hyperpolarization in the presence of norepinephrine but not in 60 mmol/L KCl.\textsuperscript{11,38} In this preparation, ACh-induced vasodilation of KCl-mediated vasoconstriction is not associated with changes in membrane potential and is abolished by N-Arg.\textsuperscript{11} In this same preparation, during NE-induced vasoconstriction, N-Arg converts the sustained ACh-induced vasodilation to a transient response but has no effect on the ACh-induced transient hyperpolarization.\textsuperscript{11} Thus, although we could not directly confirm its role in ACh-induced vasodilation in the present study, many features of the transient ACh-induced vasodilation of pressure-constricted arterioles are similar to the characteristics of smooth muscle responses to EDHF. We cannot at the present time comment on the mechanism(s) underlying this action of ACh in our model. This response does not appear to be inhibited by glibenclamide (R. Loutzenhiser, unpublished observations). However, experimental problems confound interpreting observations with other potassium channel blockers, which, by themselves potentiate vasoconstriction (eg, tetraethylammonium and 4-aminopyridine). The resolution of this issue will require direct measurements of the effects of ACh on arteriolar membrane potential during pressure-induced vasoconstriction.

Finally, additional comments are warranted concerning our interpretation that although ACh does appear to stimulate NO release in our model, this NO does not affect myogenic vasoconstriction. There is growing controversy concerning the effects of NO on renal autoregulatory responses. Our findings are consistent with a number of reports indicating that although NO synthase blockade may reduce basal renal blood flow, it does not appear to alter renal autoregulation.\textsuperscript{40-42} Others have suggested, however, that NO directly modulates myogenic reactivity\textsuperscript{43} and renal autoregulation.\textsuperscript{44,45} It may be of note that studies demonstrating modulatory effects of NO on pressure-induced afferent arteriolar vasoconstriction use juxtamedullary nephron preparations. Recently, Höffken et al\textsuperscript{46} examined regional effects of A\textsuperscript{\textdagger}O\textsuperscript{-}nitro-L-arginine methyl ester (L-NAME) on pressure-dependent arteriolar tone in the in vivo hydronephrotic kidney preparation. These authors found that L-NAME had no effect on pressure-dependent tone and autoregulation in cortical nephrons but that L-NAME treatment potentiated autoregulatory responses and increased the pressure-dependent tone of juxtamedullary nephrons. The responses of juxtamedullary vessels were not examined in the present study. Of note, it has recently been suggested that NO derived from the macula densa mediates tubuloglomerular feedback–dependent arteriolar responses.\textsuperscript{47} Tubular atrophy would eliminate any contribution of this mechanism in our preparation.

In summary, the present study demonstrates conclusively two important aspects of the renal microvascular actions of ACh. First, it is apparent from our findings that endothelial factors other than NO contribute to the renal microvascular actions of ACh. Second, the determinants of ACh-induced renal vasodilation appear to vary depending on the nature of the underlying renal vascular tone. As demonstrated previously, endothelium-derived NO may be the major factor responsible for ACh-induced vasodilation during neural- or hormonal-induced renal vasoconstriction. In contrast, other EDRFs (eg, EDHF and prostaglandins) may be more important mediators of ACh-induced vasodilation under conditions in which pressure-induced or myogenic tone is the primary determinant of basal renal vascular resistance.

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