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Cyclic GMP-dependent and cyclic GMP-independent actions of nitric oxide on the renal afferent arteriole

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1 The effects of exogenous NO and endothelial-derived NO (EDNO) on the afferent arteriole were investigated in the *in vitro* perfused hydronephrotic rat kidney. Vessels were pre-constricted with angiotensin II (0.1–0.3 nM) or KCl (30 mM). NO was infused directly into the renal artery at concentrations ranging from 30–9000 nM. ODQ (10, 30 μM) was administered to examine the effects of guanylyl cyclase inhibition. Kidneys were treated with ibuprofen (10 μM) to avoid actions of prostaglandins.

2 During angiotensin II-induced vasoconstriction, NO elicited vasodilation at concentrations of 30–900 nM (EC_{50} = 200 nM) and ODQ caused a 10 fold shift in NO-sensitivity (EC_{50} 1600 nM). During KCl-induced vasoconstriction, NO elicited a maximal dilation of 82 ± 9% at 9000 nM (EC_{50} 2000 nM) and ODQ had no effect. Thus in the presence of ODQ, the NO concentration-response curves for KCl- and angiotensin II-induced vasoconstriction were identical ($P > 0.2$).

3 To assess the possible role of cyclic GMP-independent mechanisms in the actions of EDNO, we compared the effects of L-NAME, ODQ and ODQ + L-NAME on acetylcholine-induced vasodilation. Angiotensin II reduced afferent arteriolar diameters from 16.7 ± 0.5 to 8.1 ± 0.8 microns and acetylcholine fully reversed this effect (16.9 ± 0.5 microns). ODQ restored the angiotensin II response in the presence of acetylcholine (7.1 ± 0.6 microns) and the subsequent addition of L-NAME had no further effect (6.8 ± 0.7 microns). Similarly, L-NAME alone, fully reversed the actions of acetylcholine.

4 Our findings indicate that exogenous NO is capable of eliciting renal afferent arteriolar vasodilation through both cyclic GMP-dependent and cyclic GMP-independent mechanisms. The cyclic GMP-independent action of NO did not require K^+ channel activation, as it could be elicited in the presence of 30 mM KCl. Finally, although cyclic GMP-independent effects of exogenous NO could be demonstrated in our model, EDNO appears to act exclusively through cyclic GMP.

Keywords: Nitric oxide; cyclic GMP; angiotensin II; KCl; acetylcholine; renal microcirculation; afferent arteriole; hydronephrosis

Introduction

Nitric oxide is an important modulator of the renal microvasculature. Endothelial derived NO (EDNO) is known to affect vasomotor responses of both pre-glomerular (afferent) and post-glomerular (efferent) arterioles (for review see Navar *et al*., 1996). The vascular smooth muscle actions of NO are generally considered to involve a direct activation of guanylyl cyclase and to depend on elevations in cyclic GMP. In the kidney, the renal afferent arteriolar actions of EDNO largely resemble those of arterial natriuretic peptide, a cyclic GMP-dependent vasodilator, and permeant cyclic GMP analogues (Hayashi *et al*., 1990, 1994). Nevertheless, the renal microvascular actions of NO are not fully resolved and many aspects of the control of the renal microcirculation by EDRF's are not understood (e.g., Hayashi *et al*., 1994). Recent interest has focused on the possibility that NO may act *via* both cyclic GMP-dependent and c-GMP independent mechanisms. Studies in intact tissues and in smooth muscle myocytes demonstrate that NO can have direct effects on vascular reactivity and that these actions clearly do not require cyclic GMP (Bolotina *et al*., 1994; Kanagy *et al*., 1996; Koh *et al*., 1995; Minamino *et al*.,1 997; Plane *et al*., 1996; Takeuchi *et al*., 1996).

Although the influence of NO on renal microvascular reactivity has been studied extensively, the mechanisms of NO-induced renal vasodilation and the role of cyclic GMP in the renal microvascular actions of NO have not been previously investigated. In the present study, we examined the effects of exogenously administered NO on the renal afferent arteriole using the *in vitro* perfused hydronephrotic rat kidney preparation (Loutzenhiser & Parker, 1994). The responses to NO were assessed under conditions in which basal tone was established by either angiotensin II or KCl-induced depolarization, as the latter would eliminate vasodilation mediated by K channel activation (Reslerova & Loutzenhiser, 1995). The responses to EDNO were assessed by examining the nitric oxide synthase-dependent actions of acetylcholine. Our findings indicate that whereas exogenous NO is capable of eliciting both cyclic GMP-dependent and cyclic GMP-independent actions on this vessel, EDNO acts exclusively *via* cyclic GMP.

Methods

The isolated perfused hydronephrotic kidney

The *in vitro* perfused hydronephrotic rat kidney model (Loutzenhiser & Parker, 1994) was used to examine the role of cyclic GMP in the actions of exogenous NO and

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EDNO on the renal afferent arteriole. Unilateral hydronephrosis was produced in male Sprague Dawley rats (125 g) by ligating the left ureter under halothane-induced anesthesia. Hydronephrotic kidneys were harvested after 6–8 weeks. At this stage, tubular atrophy allows direct visualization of the renal microvasculature (Steinhausen *et al.*, 1983). Rats were anesthetized with methoxyflurane, the renal artery of the hydronephrotic kidney was cannulated, and the kidney was excised for *in vitro* perfusion. During the initial cannulation and throughout the excision, kidneys were continuously perfused with medium to avoid a disruption of nutritive flow or exposure to hypoxia, ischemia, or low perfusion pressure.

The perfusing apparatus used in the present study employed a single-pass presentation of medium to the kidney (Loutzenhiser & Parker, 1994). The medium was pumped on demand through a heat exchanger to a pressurized reservoir, supplying the renal artery. Perfusion pressure was monitored within the renal artery and altered by adjusting the pressure within the reservoir. A custom designed automated pressure-controller (University of Calgary, Biomedical Instrumentation) was used to maintain perfusion pressure at 80 mmHg. Kidneys were perfused with a modified Dulbecco's modified Eagle medium (GIBCO, Grand Island, NY, U.S.A.) containing 30 mM bicarbonate, 5 mM glucose, and 5 mM HEPES (N-2-hydroxyethylpiperazine-N-2-ethane sulphonic acid, GIBCO). The perfusate was equilibrated with 95% air/5% CO_2 ($pO_2 = 150$ torr). Temperature and pH were maintained at 37°C and 7.40, respectively. Perfusate flow was monitored using a Transonic (model T106) flowmeter.

Nitric oxide administration

A fresh stock solution of NO was prepared each morning by equilibrating (100%) NO gas in degassed distilled water. A 10 fold dilution of this stock (into degassed distilled water) was prepared and analysed by chemiluminescence (O'Neill *et al.*, 1993). A 0.3 mM solution of NO was prepared and placed in a gas-tight Hamilton syringe. A gas-impermeant tube was used to connect this syringe to the perfusion line just proximal to the renal artery (<6 cm from the renal hilus). The infusion rate of NO was adjusted to the perfusate flow rate to achieve final medium NO concentrations in the range of 30–9000 nM. Compensatory adjustments were made during the infusion to maintain a constant NO level as renal perfusate flow increased. We found responses to low concentrations of NO (30–100 nM) to be more reproducible following an initial exposure to a higher NO challenge (300–900 nM), suggesting possible interactions with tissue sites proximal to the vessel under observation. To compensate for this effect, kidneys were first exposed to the higher concentration and either allowed to recover fully or immediately challenged with a lower concentration. Reproducible NO responses were observed using either approach. Based on a renal perfusate flow of 5–15 ml min^{-1} and a combined volume of the canula dead-space and renal vasculature of <0.5 ml, we estimate the transit time from the NO infusion line to the renal venous effluent to be <2–6 s. Stock solutions of NO were prepared in the absence of oxygen, conditions in which NO is extremely stable (Beckman & Koppenol, 1996). The rate of NO degradation in the presence of oxygen is directly related to $[O_2] \times [NO]^2$. At the NO and O_2 concentrations employed (0.03–9.0 μM, NO, 150 mm Hg O_2) the apparent half-life for NO is 11–100 min (Beckman & Koppenol, 1996).

Materials

Nitric oxide was purchased from Liquid Carbonic Inc. (Calgary, AB, Canada). ODQ (1H-[1,2,4] oxidiazolo [4,3-a] quinoxalin-1-one) was obtained from Tocris Cookson Inc. (Ballwin, MO, U.S.A.). Stock solutions of ODQ were prepared in ethanol the morning of each study. Angiotensin II was obtained from Sigma-Aldrich Chemical Ltd. (Oakville, ON, Canada). Ibuprophen and L-NAME were purchased from Research Biochemicals International (Natick, MA, U.S.A.). All other reagents were obtained from GIBCO (Grand Island, NY, U.S.A.).

Analysis of data

Video images were digitized (Model IVG-128, Datacube, Peabody, MA, U.S.A.) for on-line analysis. Afferent arteriolar diameters were measured by image processing as described in detail elsewhere (e.g., Loutzenhiser, 1996). Vessel segments (20–30 microns in length) were scanned automatically and the mean arteriolar diameter along the length of the segment was determined at a sampling rate of approximately 1 Hz. The mean-diameter measurements obtained during the plateau of the response were then averaged. Typically one diameter determination was derived from the mean of 100–200 individual measurements, each in turn representing the mean diameter over the length of arteriolar segment.

Throughout the text, data are expressed as the mean followed by the standard error of the mean, as an index of dispersion. The number of replicates refers to the number of afferent arterioles examined. Only one vessel was studied in each kidney preparation. Differences between means were evaluated by analysis of variance followed by Student's *t*-test (paired or unpaired). Probabilities (P) less than 0.05 were considered statistically significant.

Results

An original tracing illustrating the effect of NO infusion on angiotensin II-induced vasoconstriction is depicted in Figure 1. In this experiment 0.1 nM angiotensin II reduced afferent arteriolar diameter from approximately 18 microns to 8 microns. The infusion of NO at a rate sufficient to attain a

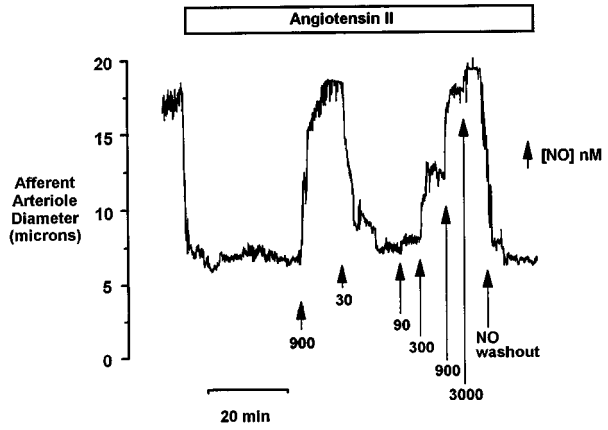

Figure 1 An original tracing illustrating the concentration-dependent vasodilatory effects of infused NO on afferent arterioles preconstricted with 0.1 nM angiotensin II. NO concentrations (nM) indicated at arrows.

perfusate concentration of 900 nM resulted in a full vasodilation. Vasoconstriction was promptly reestablished when the infusion rate was reduced (in this case NO levels were lowered to 30 nM). Subsequent increases in NO administration produced concentration-dependent vasodilation. These effects of NO were fully reversed upon cessation of the NO infusion. Figure 2 depicts paired data examining the reproducibility of NO-induced vasodilation in this preparation. In these studies arterioles were pre-constricted with angiotensin II and then exposed to two consecutive challenges with increasing concentrations of NO. Identical degrees of inhibition were observed at each NO concentration ($n=4$; $P>0.20$), indicating that over this concentration range the response to NO was fully reversible and reproducible.

Figure 3 summarizes the concentration-dependent effects of NO on angiotensin II-induced vasoconstriction in the renal afferent arteriole and the inhibition of these actions of NO by the guanylyl cyclase inhibitor ODQ (10 μM). In controls, angiotensin II (0.1 nM) reduced afferent arteriolar diameters from 16.7 ± 0.8 microns to 6.9 ± 0.4 microns ($n=10$). At 30 nM NO, afferent arteriolar diameter increased to 8.2 ± 0.3 ($n=9$; $P<0.05$, versus angiotensin alone). Increasing concentrations of NO elicited progressively greater vasodilation. At 900 nM, NO fully reversed angiotensin II-induced vasoconstriction, returning diameters to basal levels (16.0 ± 0.7 microns, $n=10$; $P>0.1$, versus pre-angiotensin II). These data are plotted as per cent inhibition in Figure 3. The IC_{50} for NO-induced vasodilation in this setting was approximately 200 nM.

In a separate series of experiments, kidneys were pre-treated with 10 μM ODQ before the administration of NO. In these studies ODQ had no effect on basal diameter (16.4 ± 1.0 microns versus 16.1 ± 1.1 microns, for control and ODQ, respectively, $n=7$; $P>0.2$). Angiotensin II constricted afferent arterioles to 6.5 ± 0.6 microns ($n=7$), a value similar to that seen in the absence of ODQ (6.9 ± 0.4 microns, $P>0.5$). In the presence of ODQ, increasing the NO concentration to 900 nM did not elicit a significant vasodilation (7.9 ± 1.5 microns, $n=7$; $P>0.4$ versus angiotensin II alone). However, when [NO] was increased to 3 μM, angiotensin II-induced vasoconstriction was completely reversed (15.8 ± 1.3 microns, $n=7$; $P>0.05$ versus pre-angiotensin II diameter). These data are plotted as percent inhibition in Figure 3. As depicted, ODQ shifted the EC_{50} by approximately 1 log unit. In rat mesenteric artery, 10 μM ODQ has been shown to fully inhibit guanylyl cyclase activity (Plane *et al*., 1996). Nevertheless, since ODQ is suggested to be a competitive inhibitor of NO-induced activation of guanylyl cyclase (Olson *et al*., 1997), 10 μM ODQ may have been submaximal in our preparation. We therefore determined if increasing ODQ to 30 μM ($n=5$) would result in a further inhibition of the actions of NO. We found 10 and 30 μM ODQ to have similar effects. Thus, 900 and 3000 nM NO inhibited angiotensin II-induced vasoconstriction by $10\pm15\%$ and $91\pm8\%$, respectively ($n=7$) in the presence of 10 μM ODQ, and by $10\pm6\%$ and $69\pm14\%$ in the presence of 30 μM ODQ ($n=5$, $P>0.10$).

We next examined the actions of NO and ODQ on the afferent arteriole when tone was established by KCl-induced depolarization (Figure 4). Constriction with 25–30 mM KCl reduced the afferent arteriolar diameter from 17.7 ± 0.9 microns to 5.9 ± 1.4 microns ($n=6$). NO did not produce significant vasodilation at concentrations below 3000 nM. At

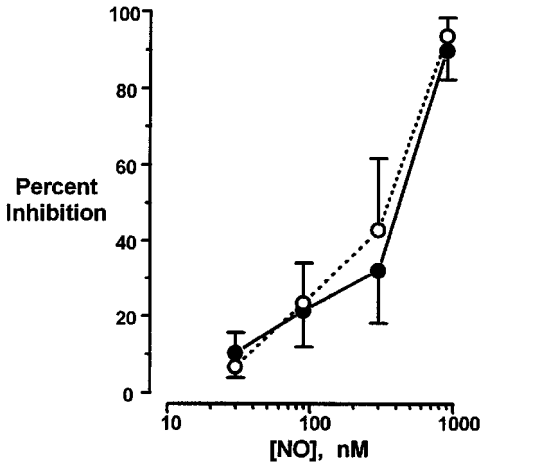

Figure 2 Reproducibility of NO-induced vasodilation. Two consecutive dose responses to NO were obtained. The first is depicted by closed circles, the second by open circles. Responses to NO are plotted as the percent inhibition of the angiotensin II-induced vasoconstriction. Means$\pm$s.e.mean ($n=4$, $P>0.2$ at all concentrations).

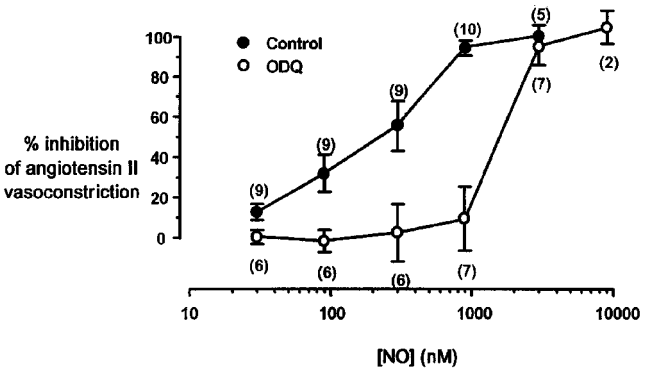

Figure 3 Concentration-dependent effects of NO on afferent arterioles preconstricted with 0.1 nM angiotensin II (closed circles). Blockade of guanylyl cyclase with 10 μM ODQ (open circles) shifted the concentration-response curve by approximately 1 log unit. Means$\pm$s.e.mean (n for each data point indicated in parenthesis).

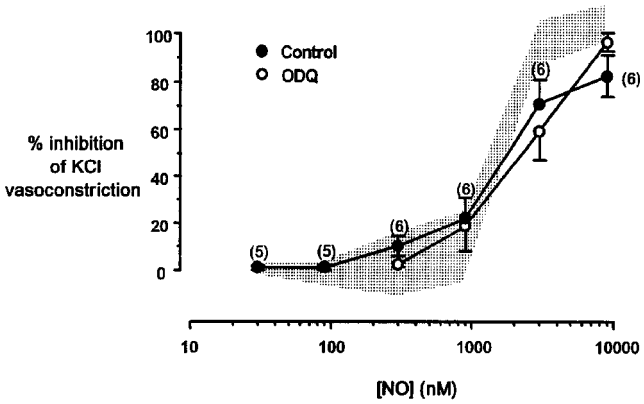

Figure 4 Concentration-dependent effects of NO on afferent arterioles preconstricted with 25–30 mM KCl (closed circles, n indicated in parenthesis). In contrast to angiotensin II-induced vasoconstriction (Figure 3), during KCl-induced vasoconstriction, blockade of guanylyl cyclase with 10 μM ODQ had no effect on the actions of NO (open circles, $n=6$, $P>0.2$ versus control). The shaded area represents data obtained for NO-induced vasodilation during angiotensin II-induced vasoconstriction in the presence of ODQ (means$\pm$s.e.mean, from Figure 3).

3000 nM, NO increased diameter to 13.8±0.9 microns ($n=6$; $P<0.005$, versus KCl alone). However, even at this concentration of NO, significant KCl-induced vasoconstriction remained ($P<0.05$ versus pre-KCl diameter, 17.7±0.9 microns). Increasing NO concentration to 9000 nM increased diameter to 15.2±0.9 microns. ($P>0.05$ versus pre-KCl). These data are plotted as percent inhibition to Figure 4. The IC_{50} was approximately 2000 nM. Thus, NO was less potent in reversing KCl- versus angiotensin II-dependent afferent arteriolar tone.

ODQ did not appear to alter the actions of NO during KCl-induced vasoconstriction. Basal afferent arteriole diameter was 16.9±0.9 microns following 10 μM ODQ ($n=6$, $P>0.4$ versus control, 17.2±1.0 microns). In this setting, 25–30 mM KCl reduced afferent arteriolar diameter to 4.4±1.4 microns ($n=6$), a value similar to that seen in the absence of ODQ (5.9±1.5 microns, $P>0.4$). NO did not elicit significant vasodilation at concentrations below 3000 nM. At 3000 nM and 9000 nM NO, diameters increased to 11.7±2.4 microns ($P<0.01$ versus KCl alone) and 16.6±0.9 microns ($P>0.4$ versus pre-KCl), respectively. At each NO concentration, diameters attained in the presence of ODQ were not significantly different from diameters attained in the absence of ODQ ($P>0.2$). These data are plotted as percent inhibition in Figure 4. The shaded area in Figure 4 depicts the means±s.e.mean of NO-induced inhibition of angiotensin II-dependent tone observed in the presence of ODQ. Note that the concentration-response curves for the cyclic GMP-independent actions of NO were similar in these two settings. In each of these studies, NO-induced vasodilation was assessed under similar levels of vasoconstriction. Table 1 summarizes the afferent arteriolar diameters observed during basal conditions and in the presence of angiotensin II or KCl for each protocol.

Table 1 Afferent arteriolar diameters (in microns)

	Control	*ODQ (10 μM)*	*ODQ (30 μM)*
Basal	16.7±0.8	16.1±1.1	15.5±0.5
Angiotensin II	6.9±0.4	6.5±0.6	7.0±0.6

	Control	*ODQ (10μM)*
Basal	17.7±0.9	16.9±0.9
KCl	5.9±1.4	4.4±1.4

The above findings suggest that exogenous NO elicits both cyclic GMP-dependent and cyclic GMP-independent actions on the afferent arteriole. We next determined if NO released by physiologic stimuli (i.e., EDNO) also elicited vasodilation by both cyclic GMP-dependent and -independent mechanisms. The tracings depicted in Figure 5 illustrate the protocols used in these experiments. In each case, basal tone was established with angiotensin II and acetylcholine-induced vasodilation was elicited in the presence of 10 μM ibuprofen. Figure 5a illustrates that, in this setting, 1.0 μM acetylcholine elicits a sustained maximal vasodilation. Application of 100 μM L-NAME reversed the actions of acetylcholine, restoring the angiotensin II-induced vasoconstriction within 5 min (Figure 5b). The application of 10 μM ODQ produced a similar effect, fully restoring angiotensin II-dependent tone (Figure 5c). Furthermore, when L-NAME was applied following ODQ-treatment to eliminate any residual actions of EDNO, no further vasoconstriction was evident. These findings suggest that ODQ fully inhibits the actions of EDNO. To assure that maximal NO release was attained in these experiments, protocols B and C were undertaken with both 1 μM and

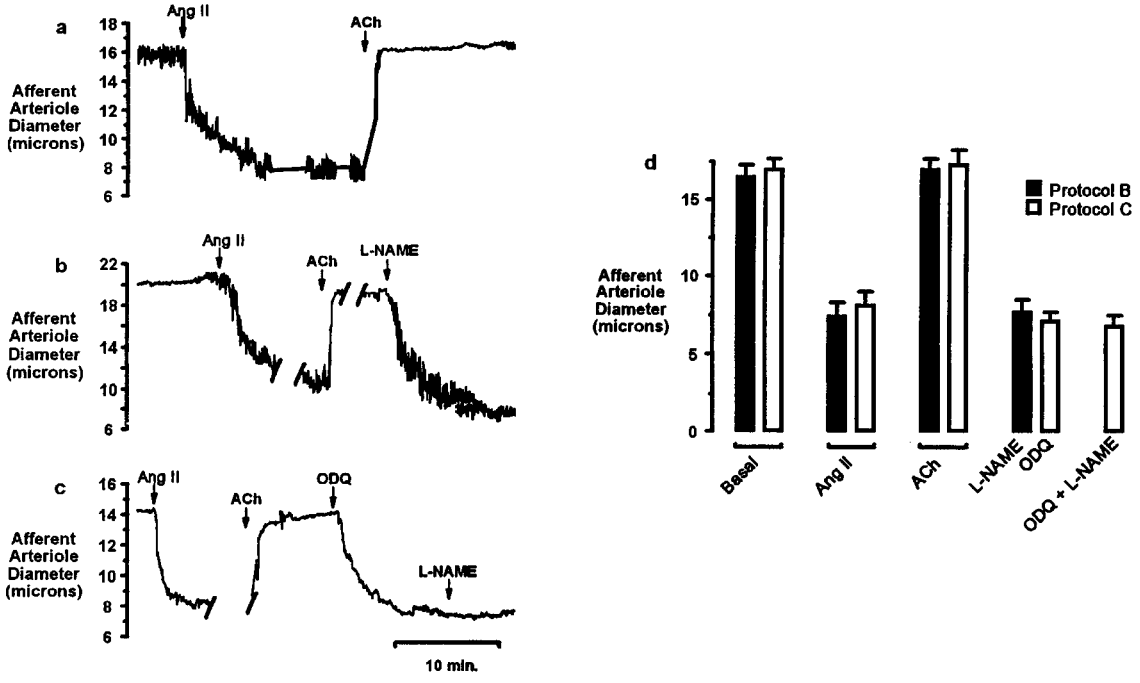

Figure 5 Effects of ODQ on EDNO-induced vasodilation. All studies conducted in presence of cyclo-oxygenase inhibition (10 μM ibuprofen). As depicted in (a) acetylcholine (ACh) elicits a sustained vasodilation of angiotensin II (Ang II, 0.1 nM) dependent afferent arteriolar tone. Blockade of nitric oxide synthase with 100 μM L-NAME completely abolished the actions of acetylcholine (b). ODQ (10 μM) also completely abolished the actions of acetylcholine and subsequent removal of NO (L-NAME) had no further effect (c). Mean data illustrating the reversal of acetylcholine-induced vasodilation by L-NAME (protocol B, $n=6$) or ODQ followed by ODQ+L-NAME (protocol C, $n=8$) are depicted in (d). Acetylcholine administered at 1.0 and 100 μM (see text).

100 μM ACh. ODQ fully restored angiotensin II-induced vasoconstriction at each concentration (124 $\pm$ 13% and 106 $\pm$ 5% of the original angiotensin II response, for 1 μM and 100 μM acetylcholine, respectively). These data were combined and are summarized in Figure 5d. Thus, although cyclic GMP-independent actions of exogenously applied NO could be demonstrated in this preparation, we found the vasodilatory actions of EDNO are mediated exclusively by cyclic GMP-dependent mechanisms. In a separate series of experiments, 100 μM L-NAME produced only a modest afferent arteriolar vasoconstriction in the absence of induced tone (control, 16.0 $\pm$ 0.5; L-NAME, 15.7 $\pm$ 0.5, $n=6$, $P<0.025$).

Finally, we examined the EDNO effects of acetylcholine on KCl-induced vasoconstrictions. Previous studies using an earlier adaptation of this model had shown that acetylcholine has minimal effects on KCl-induced afferent arteriolar vasoconstriction in the presence of cyclo-oxygenase blockade (Hayashi *et al*., 1994). In the present study 30 mM KCl reduced afferent arteriolar diameters from 16.0 $\pm$ 0.3 to 4.9 $\pm$ 0.9 microns ($n=5$). Acetylcholine (10 μM) caused a small vasodilation (to 5.9 $\pm$ 1.3 microns, $P<0.05$) and this response was reversed by ODQ (4.9 $\pm$ 0.8 microns, $P>0.25$ versus angiotensin II alone). L-NAME produced no further vasoconstriction (4.8 $\pm$ 0.8 microns).

Discussion

The present study is the first to examine the effects of exogenously applied NO on the renal microcirculation and to determine the role of cyclic GMP-dependent versus cyclic GMP-independent mechanisms. Our findings indicate that exogenous NO elicits afferent arteriolar vasodilation *via* a guanylyl cyclase-dependent mechanism over concentrations ranging from 30 nM to 900 nM. Although cyclic GMP-independent effects of exogenous NO were observed at high concentrations (1 – 9 μM), we found no evidence that an ODQ-insensitive mechanism contributes to the vasodilatory effects of endogenously released NO. Thus, our data suggest that EDNO elicits vasodilation in the afferent arteriole exclusively through a cyclic GMP-dependent mechanism.

We observed NO to elicit concentration-dependent afferent arteriolar vasodilation at concentrations of 30 – 900 nM, with an EC_{50} of approximately 200 nM. To our knowledge this is the first dose-response data for NO-induced dilation in this vessel. Previous studies assessing *in vitro* effects of aqueous solutions of NO in other vascular preparations report NO-induced vasodilation over the same concentration range (Garcia-Pascual, *et al*., 1995; Khan, Mathews & Meisheri, 1993). Similarly, measurements of NO at the endothelial surface using porphyrinic-based microsensors suggest that EDNO acts at similar concentrations. Thus, Malinski & Taha (1992) found bradykinin elevates NO to 450 $\pm$ 40 nM in the porcine aorta. Gerová *et al*. (1996) found acetylcholine increases luminal NO concentrations of the canine femoral vein to 360 $\pm$ 50 nM, *in vivo*. Similarly, Cohen *et al*. (1997) found acetylcholine increased local NO concentrations from $<$10 nM to approximately 300 nM in rabbit carotid artery segments. Given the technical constraints of this approach and the assumption that the values measured at the endothelial surface would underestimate the NO at the smooth muscle-endothelium interface, our data agree closely with these observations.

A number of studies demonstrate that exogenous NO is capable of altering smooth muscle tone by mechanisms that do not depend on the activation of guanylyl cyclase or elevation of cyclic GMP. Bolotina *et al*. (1994) found NO applied to excised membrane patches from rabbit aortic smooth muscle cells activated a 250 – 300 pS charybdotoxin-sensitive K channel, and suggested that NO directly activates large conductance Ca-activated K^+ channels (BK) by nitrosylation. George & Shibata (1995), Li *et al*., (1997) and Koh *et al*. (1995) also observed that the direct application of NO activated BK, in rabbit and bovine coronary artery, and in canine colon myocytes. In contrast toBolotina *et al*. (1994). these authors found this action of NO required cyclic GMP, was blocked by ODQ (Li *et al*., 1997) and was mimicked by permeant cyclic GMP analogues. Koh *et al*. (1995) did observe NO to activate smaller K^+ channels (4 pS and 80 pS) in colonic smooth muscle *via* a cyclic GMP-independent mechanism. Finally, Plane *et al*. (1996) found the relaxant effects of the NO donor SIN-1 on the rat mesenteric artery were relatively insensitive to ODQ and suggested that a charybdotoxin-sensitive cyclic GMP-insensitive mechanism contributes to the actions of NO in this preparation.

The present study was the first to examine the role of cyclic GMP in the actions of NO on the renal afferent arteriole. We found ODQ pre-treatment to cause a 10 fold shift in the sensitivity of this vessel to NO during angiotensin II-induced vasoconstriction. Nevertheless, full NO-induced vasodilation was observed in the presence of 10 μM ODQ. This concentration of ODQ is reported to fully prevent NO-induced activation of guanylyl cyclase (Plane *et al*., 1996, Olson *et al*., 1997), and even higher concentrations of ODQ (30 μM) did not fully block NO-induced vasodilation in our studies. Interestingly, ODQ had no effect on NO-induced afferent arteriolar vasodilation in vessels pre-constricted with KCl, suggesting NO-induced activation of guanylyl cyclase has minimal effects on this response, at least in the absence of prostaglandins (ibuprofen was present in our studies). It is well-known that cyclic GMP is more efficacious against agonist-versus KCl-induced vasoconstriction (e.g., Winquist *et al*., 1984), and early studies demonstrated that EDNO is relatively ineffective against KCl-induced vasoconstriction (e.g., Furchgott & Zawadzki, 1980). However, the actions of NO may depend, in part, on the experimental setting, as previous studies with our model indicate that EDNO and prostaglandins act in a synergistic manner to attenuate KCl-induced vasoconstriction (Hayashi *et al*., 1994).

The cyclic GMP-insensitive vasodilatory actions of NO were equally effective against KCl- and angiotensin II-induced vasoconstriction (Figure 4). The effects of altered K^+ conductance are minimized when extracellular [K^+] is elevated and we have previously demonstrated that K channel activation by either pinacidil (Reslerova & Loutzenhiser 1995) or hypoxia (Loutzenhiser & Parker, 1994) does not alter KCl-induced vasoconstriction in this model. Thus, our findings do not support a K channel dependent mechanism. Unlike the ODQ-sensitive responses observed at lower concentrations (Figure 1), the effects of high concentrations of NO were often irreversible (data not shown). Kanagy *et al*. (1996) found that pre-treatment of tissues with the NO donor S-nitroso-N-acetylpenicillamine (SNAP) produced a long-lasting inhibition of KCl-induced vasoconstriction *via* a cyclic GMP-independent mechanism and suggested that this may reflect an irreversible covalent modification of signaling proteins, such as L-type Ca channels. Alternatively, at high concentrations, NO may produce cytotoxic effects. Micromolar levels of NO inhibit the glycolytic enzyme glyceraldehyde 3-phosphate dehydrogenase (Brüne *et al*., 1994) and block oxidative phosphorylation (Cleeter *et al*., 1994). Xu *et al*. (1997) demonstrated that NO treatment induces the expression of a

heat-shock protein (hsp70) indicating a general activation of the stress response in vascular smooth muscle cells. Finally, in the presence of oxygen, elevated concentrations of NO may lead to toxic levels of peroxynitrite (Beckman & Koppenol, 1996).

Although our study demonstrates that exogenous NO is capable of eliciting vasodilation *via* a cyclic GMP-independent mechanism, EDNO appeared to act exclusively through cyclic GMP. Previous studies with our model have shown that acetylcholine elicits afferent arteriolar vasodilation *via* EDNO, prostanoids and an unidentified factor, presumed to be an endothelium-derived hyperpolarizing factor (EDHF, Hayashi *et al*., 1994). EDHF acts transiently in this vessel and the sustained vasodilator response to acetylcholine is predominantly due to EDNO (Hayashi *et al*., 1994). Similarly in the present study, L-NAME completely blocked the sustained acetylcholine-induced vasodilation in the presence of ibuprofen. This EDNO component was also fully inhibited by ODQ, suggesting that EDNO acts exclusively *via* cyclic GMP.

The reasons why EDNO, unlike endogenous NO, did not exhibit a cyclic GMP-independent component are not clear. It is possible that EDNO does not attain the high concentration required to elicit the ODQ-insensitive NO-induced vasodilation. As discussed above, measurements of EDNO during exposure of endothelium to acetylcholine or bradykinin indicate NO levels reach 300–450 nM (Malinski *et al*., 1992; Cohen *et al*., 1997). We did not observe cyclic GMP-independent actions of NO at concentrations below 900 nM. However, the NO concentration at the endothelium/smooth muscle interface may reach concentrations much higher than that measured at the endothelial surface. Furthermore, micromolar levels of NO may be attained during pathophysiologic conditions. For example, Malinski *et al*. (1993) found that during cerebral ischemia, tissue levels of NO increase from approximately 10 nM to >2 μM.

Alternatively, it is possible that the route of administration of NO may influence the actions of NO. NO has direct effects on the endothelium (Ma *et al*., 1996) and differential responses to luminal versus adluminal application of NO have been observed (Steinhorn *et al*., 1995). Thus, EDNO released at the smooth muscle/endothelium interface and exogenous NO administered to the vessel lumen may have differing effects. Finally, it is conceivable that a factor related to NO, but not NO itself mediates the NOS-dependent component of acetylcholine-induced vasodilation (Myers *et al*., 1990).

In conclusion, the present study demonstrates that NO is capable of eliciting renal afferent arteriolar vasodilation by both cyclic GMP-dependent and cyclic GMP-independent mechanisms. The cyclic GMP-independent effects of NO occurred at concentrations above 1 μM and affected angiotensin II and KCl-induced vasoconstriction in a similar manner, suggesting a mechanism other than K^+ channel activation. Although cyclic GMP-independent actions of exogenous NO were observed in our study, we found that EDNO released by acetylcholine acts exclusively through cyclic GMP. Thus, the cyclic GMP-independent mechanism appears to play a minor role in EDNO-mediated vasodilation in the renal afferent arteriole.

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