Contribution of 20-HETE to the vasodilator actions of nitric oxide in renal arteries

MAGDALENA ALONSO-GALICIA,1 CHENG-WEN SUN,1 JOHN R. FALCK,2
DAVID R. HARDER,1 AND RICHARD J. ROMAN1

1Department of Physiology, Medical College of Wisconsin,
Milwaukee, Wisconsin 53226; and 2Department of Molecular Genetics,
University of Texas Southwestern Medical Center, Dallas, Texas 75235

A recent study examined the contribution of elevations in cGMP versus inhibition of cytochrome P-450A enzymes and the production of the vasoconstrictor 20-hydroxyeicosatetraenoic acid (20-HETE) to the vasodilator actions of NO in renal arterioles. The NO donor sodium nitroprusside (SNP) at 10-3, 10-4, and 10-5 M reduced the production of 20-HETE in microsomes prepared from renal arteries to 80 ± 2, 43 ± 5, and 7 ± 1% of control, respectively (n = 4). Furthermore, the vasodilator response to SNP (10-7 to 10-3 M) was examined in rat renal interlobular arteries (≈90 µm ID), preconstricted with phenylephrine (1 µM) under control conditions and after blockade of the cGMP and P-450A pathways. Inhibition of guanylyl cyclase with 1H-1,2,4-oxadiazole[4,3-a]quinoxalin-1-one (ODQ) (10 µM, n = 6) or of cGMP-dependent protein kinase with 8R,9S,11S-(-)-9-methoxy-carbamyl-8-methyl-2,3,9,10-tetrahydro-8,11-epoxy-1H,8H,11H-2,7-b,11a-triazidibenzo(a,g)-cycloocta-(c,d,e)-trinden-1-one (KT-5823, 1 µM; n = 5) markedly impaired the vasodilator response to SNP by 26 and 30%, respectively. In contrast, inhibition of the endogenous production of 20-HETE with a suicide substrate, irreversible inhibitor [17-octadecynoic acid (17-ODYA), 1 µM, n = 5], or a selective, competitive inhibitor of 20-HETE formation (dibromo-dodeceny1-methylsulfinamide, 25 µM, n = 5) markedly impaired the vasodilator response to SNP by 76 and 78%, respectively. Similarly, when 20-HETE levels were fixed at 100 nM (n = 6), the response to SNP was attenuated by 73%. Blockade of both pathways with ODQ and 17-ODYA completely abolished the response to SNP (n = 6). These results indicate that the vasodilator response to NO is largely cGMP independent and that inhibition of 20-HETE formation contributes to the cGMP-independent effects of NO in the renal microcirculation.

Numerous studies have indicated that NO plays a major role in the regulation of renal vascular tone and tubuloglomerular feedback (2, 17). In general, NO is thought to relax smooth muscle cells by binding to the heme moiety of soluble guanylyl cyclase and increasing levels of cGMP (2, 17). Elevations in cGMP activate cGMP-dependent protein kinase, which in turn phosphorylates proteins that increase the extrusion of calcium from the cell, increase reuptake of Ca2+ into the sarcoplasmic reticulum, decrease the sensitivity of the contractile mechanism to Ca2+ and activate K+ channels, which hyperpolarize the cell and limit Ca2+ influx through voltage-sensitive channels (2, 7, 17). However, this generalized scheme for NO-induced vasodilation has recently been questioned, since there are an increasing number of reports showing that NO donors and/or endothelial-dependent dilators can still produce varying degrees of vasodilation and/or hyperpolarization in some vessels in the presence of guanylyl cyclase inhibitors (5, 8, 21, 22, 27). There are also reports that the vasodilator response to NO can be dissociated from changes in vascular cGMP levels (3, 7).

In the renal microcirculation, the mechanisms by which NO or endothelial-dependent dilators alter vascular tone are not as well understood (23). It has not been directly determined whether NO actually increases cGMP levels in renal arterioles and to what extent cGMP- or cGMP-independent pathways contribute to the vasodilator response to NO. In view of recent evidence that NO is a highly reactive molecule that inhibits NO synthase (10, 12) and P-450 enzymes of the 1A and 2B1 (26) and 3C (15) families by forming iron-nitrosyl complexes, we also considered an alternative hypothesis that NO might inhibit the formation of 20-hydroxyeicosatetraenoic acid (20-HETE) in renal vascular smooth muscle and that this might contribute to the vasodilator actions of NO. Therefore, the purpose of the present study was to determine whether NO inhibits the formation of 20-HETE via cytochrome P-450 enzymes in renal arterioles and to determine the relative contribution of elevations in cGMP levels versus inhibition of 20-HETE formation to the vasodilator response to NO in the renal microcirculation.

Methods

General. Experiments were performed on 10- to 12-wk-old male Sprague-Dawley rats purchased from Harlan Sprague-Dawley Laboratories (Indianapolis, IN). The rats were housed in the animal care facility at the Medical College of Wisconsin, which is approved by the American Association for the Accreditation of Laboratory Animal Care, and the animals had free access to food and water. All protocols involving animals received approval by the Animal Care Committee of the Medical College of Wisconsin.

Isolation of renal microvessels. Adult male rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (50 mg/kg body wt), and the abdominal aorta below the renal arteries was cannulated. The aorta above the renal arteries was ligated, and the kidneys were flushed with 20 ml of low-calcium solution containing 145 mM NaCl, 6 mM KCl, 4.2 mM NaHCO3, 1 mM MgCl2, 50 µM CaCl2, 10 mM HEPES, and 10 mM glucose, followed by perfusion of 30 to 40 ml of low-calcium solution containing 6% BSA and 1% Evans blue to facilitate identification of preglomerular vessels. The kid-
ney sections, and forced through a wire-mesh sieve (150 µm) using the plunger of a 30-ml syringe. The material retained on top of the mesh screen was incubated in a low-calcium solution containing 18.1 U/ml collagenase type II, 6.5 mM dithiothreitol, 0.05 mM soybean trypsin inhibitor, and 0.1% BSA for 20 min at 37°C to remove tubular tissue. The incubation was superfused with O2 to maintain the viability of the vessels. The microvessel suspension was then filtered through a 70-µm nylon mesh and rinsed several times with low-calcium solution. The microvessels retained on the nylon mesh (40 to 150 µm ID) were then collected using a dissecting microscope and used for biochemical analysis of the metabolism of arachidonic acid (AA) via the cytochrome P-450 pathway and measurement of cGMP levels.

Effects of NO donors on the renal metabolism of AA. Preglomerular arteries were homogenized in a 10 mM potassium phosphate buffer (pH 7.7) containing 250 mM sucrose, 1 mM EDTA, and microsomes were prepared by differential centrifugation as we have previously described (20). P-4504A enzyme activity was assayed by incubating the microsomes (0.5 mg protein) for 30 min at 37°C with [1-14C]AA (0.1 µCi; 10 µm/l) in 1 ml of a 100 mM potassium phosphate buffer (pH 7.4) containing 5 mM MgCl2, 1 mM EDTA, and microsomes were prepared by differential centrifugation (11,000 rpm for 20 min at 4°C). The pellets were resuspended in 1 ml of buffer, and cGMP levels were determined in 100-µl aliquots using a commercially available RIA kit (PerSeptive, Cambridge, MA). The range of the standard curve was 2 to 128 fmol/tube with a Bo of 39.6 ± 2.5% and B50 of 9.4 ± 1.2%. Repeated analysis of a pooled vessel sample averaged 1,610 ± 80 fmol/tube with a coefficient of variation of less than 5% (n = 9).

Isolated vessel studies. Arterial and interlobular arteries of 50–100 µm inner diameter were microdissected from the kidneys of rats. The vessels were mounted on glass micropetites in a perfusion chamber in PSS equilibrated with a 95% O2:5% CO2 gas mixture and maintained at 37°C. The vessels were preconstricted with phenylephrine (1 µM) under control conditions and after addition of various concentrations of sodium nitroprusside (SNP; 10-5, 10-4, and 10-3 M) or the non-cyanide-releasing NO donor 1-propanamine, 3-(2-hydroxy-2-nitroso-1-propylhydrazino) (PAPA NONOate, 10-4 and 10-3 M). The reactions were terminated by acidification to pH 4 using 0.1 M formic acid followed by extraction with ethyl acetate. Metabolites were separated using a reverse-phase HPLC column (Supelco, Bellefonte, PA) and a linear elution gradient ranging from acetonitrile/water/acetic acid (50:50:0.1) to acetonitrile/acetic acid (100:0.1) over a 40-min period. The radioactive products were monitored using a radioactive flow detector (model 120; Radiomatic Instrument, Tampa, FL).

Measurement of NO concentration using the oxyhemoglobin trapping technique. The concentration of NO produced by addition of SNP to the bath of isolated vessels was measured using the oxyhemoglobin trapping technique. The method is based on the reaction of oxyhemoglobin with NO to form methemoglobin and nitrate (9). SNP (10-3 and 10-4 M) was added to physiological salt solution (PSS), saturated with 95% O2 and 5% CO2 at 37°C. Aliquots of the bath were collected after 3 min when the maximum vasodilator effects of SNP were recorded in the isolated vessel studies. The samples were added to 1 ml of an oxyhemoglobin solution (1 µM in 50 mM phosphate-buffered saline, pH 7.4), and visible-light difference spectra were recorded over a range in wavelength from 350 to 450 nm using a scanning spectrophotometer (model DU-620; Beckman Instruments, Fullerton, CA). The difference in absorbance between 401 and 419 nm of the sample and an oxyhemoglobin blank was calculated, and the NO concentration in the cuvette was calculated using an extinction coefficient of 100 mM-1·cm-1·mol-1 (9).

Effect of NO donors on cGMP production in renal microvessels. Renal microvessels (5–10 mg protein) were preincubated for 30 min at 37°C in 5 ml PSS containing (in mM) NaCl, 4.7 KCl, 1.17 MgSO4, 1.6 CaCl2, 12 NaHCO3, 1.18 NaH2PO4; 0.03 EDTA, and 10 glucose, pH 7.4, on a shaking water bath. The microvessels were then transferred to glass vials containing 1 ml of PSS, 0.5 mM IBMX, and various concentrations of SNP (10-5 to 10-3 M) in the presence of vehicle or 10 µM 1H-[1,2,4]oxadiazolo[4,3-a]quinolin-1-one (ODQ). The samples were incubated for 10 min at 37°C with constant shaking. The reaction was terminated by adding trichloroacetic acid to a final concentration of 6%. The samples were homogenized and centrifuged at 11,000 rpm for 20 min at 4°C, and the supernatant was extracted four times with 5 vol of water-saturated ether. The aqueous phase was collected, dried under nitrogen, and stored at −80°C until assay. The pelicit was resuspended in 1 ml of 1 N NaOH, and protein content was determined using the Bradford dye-binding procedure (Bio-Rad, Hercules, CA) for normalization of cGMP levels per milligram of tissue protein. The dried samples were reconstituted in 1 ml of buffer, and cGMP levels were determined in 100-µl aliquots using a commercially available RIA kit (PerSeptive, Cambridge, MA). The range of the standard curve was 2 to 128 fmol/tube with a Bo of 39.6 ± 2.5% and B50 of 9.4 ± 1.2%. Repeated analysis of a pooled vessel sample averaged 1,610 ± 80 fmol/tube with a coefficient of variation of less than 5% (n = 9).

To determine the contribution of cGMP to the vasodilator effects of NO, the response to SNP (10-7 to 10-3 M) was examined in vessels preconstricted with phenylephrine (1 µM) under control conditions and after addition of the guanlylcyclase inhibitor ODQ (10 µM) or the cGMP-dependent protein kinase inhibitor KT-5823 (1 µM) to the bath. The effectiveness of the blockade of guanylyl cyclase with ODQ was assessed by measuring the effects of ODQ on cGMP levels in renal microvessels incubated in the presence of various concentrations of SNP. The effectiveness of the blockade of cGMP-dependent protein kinase with KT-5823 was assessed by examining the effects of this compound on the vasodilator response to 8-bromo-cGMP (10-8 to 10-4 M).
RESULTS

Effect of NO donors on 20-HETE production. The results of these experiments are summarized in Fig. 1. Under control conditions (Fig. 1A), microsomes prepared from renal microvessels produced 14.15, 11.12, and 8.9 dihydroxyeicosatetraenoic acids (DiHETEs; retention times, 7, 8, and 8.5 min, respectively) and 20-HETE (retention time, 10 min) when incubated with AA. Addition of 10^{-3} M SNP to the incubation media completely blocked the formation of both 20-HETE and DiHETEs (Fig. 1B). The effects of SNP on the renal metabolism of AA were concentration dependent. SNP at concentrations of 10^{-3}, 10^{-4}, and 10^{-5} M lowered the production of 20-HETE by renal vessel microsomes to 80 ± 2, 43 ± 5, and 7 ± 1% of control, respectively (Fig. 1C). Similar effects were seen using the non-cyanide-releasing NO donor PAPA NONOate.

The contribution of cGMP to the dilator response to SNP in isolated perfused renal arterioles. The contribution of cGMP to the vasodilator response to NO donor was determined by comparing the concentration-dependent dilation to SNP under control conditions and after blocking guanylyl cyclase with ODQ (10 µM). These results are summarized in Fig. 2. The control inner diameter of these vessels averaged 91 ± 4 µm (n = 6 vessels, 6 rats). Phenylephrine (1 µM) reduced the inner diameter by ~50% to 42 ± 2 µm. SNP (10^{-7} to 10^{-5} M) increased the diameter of the vessels in a concentration-dependent manner to a maximum of 79 ± 4% of control (Fig. 2A). After blockade of guanylyl cyclase with 10 µM ODQ, the vasodilator response to SNP was moderately impaired. The inner diameter of these vessels increased to a maximum of 58 ± 4% of...
control in response to the highest (10^{-3} M) concentration of SNP. To test the effectiveness of ODQ in blocking guanylyl cyclase activity, the effect of this compound on cGMP levels was measured in renal microvessels treated with SNP. The results of these experiments are summarized on Fig. 2B. SNP (10^{-6} to 10^{-3} M) increased cGMP levels in renal vessels in a concentration-dependent manner to a maximum of 6,108 ± 1,464 fmol/mg protein. However, after blockade of guanylyl cyclase with 10 µM ODQ, cGMP production was significantly reduced on the average by 90% at every concentration of SNP tested.

The effects of blocking cGMP-dependent protein kinase with KT-5823 on the vasodilator response of NO in isolated perfused renal arterioles are presented in Fig. 3. The control inner diameter of these vessels averaged 90 ± 5µm (n = 5 vessels, 5 rats). Phenylephrine (1 µM) reduced the inner diameter by ∼50% to 42 ± 3µm. SNP (10^{-7} to 10^{-3} M) increased the diameter of these vessels to a maximum of 70 ± 9% of control (Fig. 3A). After blockade of cGMP-dependent protein kinase with 1 µM KT-5823, the vasodilator response to SNP was attenuated at the highest dose of SNP (10^{-3} M) by 31%. The effectiveness of blockade of cGMP-dependent protein kinase was assessed by measuring the effects of KT-5823 on the vasodilator response to various concentrations of 8-bromo-cGMP (10^{-6} to 10^{-4} M). The cGMP agonist 8-bromo-cGMP increased the inner diameter of renal arterioles in a concentration-dependent manner to a maximum of 70 ± 11% of control (Fig. 3B). After blockade of cGMP-dependent protein kinase with 1 µM KT-5823, the vasodilator response to 8-bromo-cGMP was completely blocked at every concentration studied.

To rule out the possibility that the vasodilator response to SNP in the presence of cGMP inhibitors was due to tachyphylaxis or to a time-dependent fall in the responsiveness of the preparation, we also performed time control experiments. In five vessels, the concentration-response curves to SNP were identical under control conditions and 30 min after administration of vehicle to the bath (data not shown).

Contribution of 20-HETE to the dilator response to SNP in isolated perfused renal arterioles. The contribution of 20-HETE to the vasodilator response to SNP was determined by comparing the concentration-dependent dilation to SNP before and after blocking the endogenous production of 20-HETE. The effects of DDMS (25 µM), a selective inhibitor of 20-HETE formation, on the vasodilator response to SNP are shown in Fig. 4A. The control inner diameter averaged 92 ± 5 µm (n = 5 vessels, 5 rats). Phenylephrine (1 µM) reduced the diameter by ∼50% to 46 ± 5µm. SNP (10^{-7} to 10^{-3} M) increased the diameter of these vessels in a concentration-dependent manner to a maximum of 83 ± 4% of control. After inhibition of 20-HETE production with DDMS, the vasodilator response to SNP was attenuated by 76%. The inner diameter of these vessels only increased to 16 ± 4% of control in response to the highest dose of SNP. Similar effects were obtained when 20-HETE production was blocked using a chemically and mechanistically different inhibitor of 20-HETE formation, 17-ODYA (1 µM). These results are presented in Fig. 4A. The control inner diameter of these vessels was 85 ± 7µm (n = 5 vessels, 5 rats). Phenylephrine (1 µM) reduced the diameter of these vessels by ∼50% to 43 ± 4µm, and SNP (10^{-7} to 10^{-3} M) increased the diameter in a concentration-dependent manner to a maximum of 90 ± 7% of control. After inhibition of 20-HETE production with 17-ODYA, the vasodilator response to SNP was attenuated by 78%.
Fig. 3. A: cumulative concentration-response curves depicting the effects of SNP on the inner diameter of isolated, perfused rat renal arterioles preconstricted with phenylephrine (1 µM) before and after blocking cGMP-dependent protein kinase. Paired experiments were performed in renal vessels under control conditions and after blocking cGMP-dependent protein kinase with 1 µM 8R,9S,11S(-)-9-methoxy-carbamyl-8-methyl-2,3,9,10-tetrahydro-8,11-epoxy-1H,11H-2,7b,11a-trizadibenzo-(a,g)-cycloocta-(c,d,e)-trinden-1-one (KT-5823) in the bath. Results are expressed as percent of control diameter after preconstriction with phenylephrine. Values are means ± SE obtained from 5 vessels obtained from 5 different rats. *Significant difference (P < 0.05) from control values.

B: effect of various concentrations of 8-bromo-cGMP on the vasodilator response to SNP in isolated, perfused rat renal arterioles preconstricted with phenylephrine (1 µM). Paired experiments were performed in renal vessels under control conditions and after addition of the cGMP-dependent protein kinase inhibitor KT-5823 (1 µM) to the bath. Results are expressed as percent of control diameter after preconstriction with phenylephrine. Values are means ± SE obtained from 3 vessels obtained from 3 different rats. *Significant difference (P < 0.05) from control values.

Fig. 4. A: cumulative concentration-response curves showing the effects of SNP on the inner diameter of isolated, perfused rat renal arterioles preconstricted with phenylephrine (1 µM) before and after blockade of the P-4504A pathway. Paired experiments were performed in renal vessels under control conditions and after blocking P-4504A activity with 2 different compounds, dibromo-dodecenyl-methylsulfimide (DDMS, 25 µM; n = 5 vessels obtained from 5 different rats) and 17-octadecylic acid (17-ODYA, 1 µM; n = 5 vessels obtained from 5 different rats), and after preventing the fall in 20-HETE levels by adding exogenous 20-HETE at a concentration of 100 nM in the bath (n = 6 vessels obtained from 5 different rats). The concentration of NO measured 3 min after addition of 10⁻⁴ and 10⁻³ M SNP to oxygenated bath solution is also shown. Results are expressed as percent of control diameter after preconstriction with phenylephrine. Values are means ± SE. *Significant difference (P < 0.05) from control values.

B: cumulative concentration-response curves depicting the effects of SNP on the inner diameter of isolated, perfused rat renal arterioles preconstricted with phenylephrine (1 µM) before and after simultaneous blockade of the cGMP and P-4504A pathways. Paired experiments were performed in renal microvessels under control conditions and after addition of the guanylyl cyclase inhibitor ODQ (10 µM) and the suicide substrate P-450 inhibitor 17-ODYA (1 µM) to the bath. Results are expressed as percent of control diameter after preconstriction with phenylephrine. The concentration of NO measured 3 min after addition of 10⁻⁴ and 10⁻³ M SNP to oxygenated bath solution is also shown. Values are means ± SE obtained from 5 vessels obtained from 5 different rats. *Significant difference (P < 0.05) from control values.
Vessel diameter only increased to 19 ± 3% of control in response to the highest dose of SNP.

The dilator response to SNP was also significantly impaired by preventing the fall in 20-HETE levels by adding a high concentration of 20-HETE (100 nM) to the bath. The results of these experiments are also presented in Fig. 4A. The control inner diameter of these vessels averaged 86 ± 6 µm (n = 6 vessels, 5 rats). Phenylephrine (1 µM) reduced the inner diameter by ~50% to 40 ± 4 µm, and SNP (10⁻⁷ to 10⁻³ M) increased the diameter in a concentration-dependent manner to a maximum of 69 ± 4% of control. After fixing 20-HETE levels, vascular diameter only increased to 23 ± 4% of control in response to 10⁻³ M SNP.

Simultaneous blockade of both pathways with 1 µM ODAQ and 1 µM 17-ODYA completely abolished the response to SNP (Fig. 4B, n = 5 vessels, 5 rats). In these experiments, SNP (10⁻⁷ to 10⁻³ M) increased the diameter in a concentration-dependent manner to a maximum of 77 ± 3% during the control period. However, after blockade of both cGMP and P₄₅₀ pathways, the vessel diameter only increased by 5 ± 4% of control in response to the highest dose of SNP.

Effects of P₄₅₀ inhibitors and Kₖₐ channel activators on baseline diameter of denuded renal arterioles. The effects of the NO donor SNP, the P₄₅₀ inhibitors DDMS and 17-ODYA, and the calcium-activated potassium-channel (Kₖₐ) activator NS-1619 on baseline diameters of phenylephrine-constricted renal arterioles are presented in Fig. 5. Baseline diameter of renal arterioles with an intact endothelium averaged 90 ± 5 µm (n = 5 vessels, 5 rats), and it was reduced to 46 ± 3 µm by phenylephrine. SNP (10⁻³ M) increased inner diameter by 69 ± 8% (Fig. 5A) in intact renal vessels preconstricted with phenylephrine. In contrast, DDMS and 17-ODYA had no significant effect on the diameter of preconstricted renal arterioles with an intact endothelium. Baseline diameter of renal arterioles in which the endothelium was removed averaged 115 ± 18 µm (n = 6 vessels, 6 rats), and it was reduced to 60 ± 9 µm by phenylephrine (1 µM). Addition of acetylcholine (1 µM) to the bath had no significant effect on the diameter of these vessels (63 ± 9 µm). SNP (10⁻³ M) increased the diameter of the vessels to 97 ± 12 µm. Blockade of 20-HETE formation for 10 min with DDMS (25 µM) produced a 42 ± 4% increase in inner diameter (n = 6) of phenylephrine-constricted vessels. Similar effects were observed when 20-HETE formation was blocked with 17-ODYA (1 µM). Similarly, activating Kₖₐ channels with NS-1619 (100 µM) produced a 49 ± 3% increase in inner diameter of phenylephrine-constricted renal vessels in which the endothelium was removed.

Measurement of NO generated by SNP. The concentration of NO in the bath averaged 260 ± 20 nM (n = 3) 3 min after addition of 10⁻³ M SNP to the oxygenated bath solution used in the isolated vessel experiments. Similarly, the concentration of NO measured in the bath averaged 60 ± 10 nM (n = 3) 3 min following addition of 10⁻⁴ M SNP to the bath.

**DISCUSSION**

The mechanism by which NO alters tone in the renal microvasculature is not well understood (23). The extent to which NO increases the production of cGMP in renal arterioles and the relative contribution of cGMP versus “cGMP-independent” signaling pathways to the vasodilator actions of NO have not been determined. Previous studies have demonstrated that NO is a highly reactive molecule that binds to and inhibits several heme-containing enzymes including NO syn-
The relative contribution of cGMP-dependent versus cGMP-independent pathways to the renal vasodilator actions of NO were also evaluated in the present study. The contribution of the cGMP pathway to the vasodilator effects of NO was assessed by comparing the response of renal arterioles to increasing amounts of SNP (10^{-7} to 10^{-3} M) before and after blocking soluble guanylyl cyclase activity with ODQ (10 μM). After ODQ, the vasodilator response to SNP in the range of concentrations from 10^{-6} to 10^{-4} M was not statistically significantly different from control. Only at the highest dose of SNP (10^{-3} M) was the response moderately impaired (by ~25%). The inability of ODQ to block the vasodilator response to SNP was not due to a failure to block guanylyl cyclase activity in these vessels. Indeed, we found that SNP (10^{-3} M) produced a sixfold increase in cGMP levels in renal microvessels under control conditions, and 10 μM ODQ completely blocked the ability of this high dose of SNP to increase cGMP levels.

Further evidence that the vasodilator response to SNP in the renal circulation is cGMP independent was derived from experiments using the cGMP-dependent protein kinase inhibitor KT-5823. This compound had similar effects as ODQ and attenuated the response to SNP only at the highest dose studied (10^{-3} M) by ~30%. Again, the lack of effect of KT-5823 to block the vasodilator effect of SNP was not due to an incomplete blockade of cGMP-dependent protein kinase activity, since KT-5823 completely blocked the vasodilator response to 8-bromo-cGMP at the same concentration studied. Together with the ODQ data, these results indicate that the renal vasodilator response to NO is largely cGMP independent.

Several recent observations indicate that NO binds to heme and inhibits NO synthase and other P-450 enzymes (10, 12, 15, 26). In the present study, we found that NO donors inhibit the formation of 20-HETE, a powerful vasoconstrictor in renal microvessels. Previous studies have also indicated that renal vascular smooth muscle (VSM) cells metabolize AA via a P-4504A-dependent pathway to 20-HETE and that this system serves as a novel signal transduction pathway that plays an important role in the regulation of renal vascular tone (1, 11, 13, 14, 29–31). 20-HETE is a powerful constrictor (EC_{50} < 10^{-9} M) of renal and cerebral arterioles (11, 13). It promotes Ca^{2+} entry by depolarizing these vessels secondary to blockade of the K_{Ca} channels (29). The findings that the vasodilator actions of 20-HETE in the afferent arteriole of the rat are mimicked by the blockade of K_{Ca} channels and that the vasodilator actions of 20-HETE can be blocked by iberiotoxin and tetracylammonium chloride, but not by 4-aminopyridine (a blocker of the delayed rectifier K^{+} channel), support this mechanism of action (29).

Based on these observations, we next studied the effect of blockade of the P-450 pathway on the dilator response to SNP in renal microvessels. Inhibition of the production of 20-HETE with the irreversible P-450 inhibitor, 17-ODYA, markedly impaired the response to SNP by 76%. Identical effects were seen using the chemically and mechanistically different P-450 inhibitor, DDMS. These results argue against the possibility that the attenuating effect of these compounds on the vasodilator response to SNP was due to a nonspecific action. Moreover, we have previously reported that DDMS does not alter the response to NO-independent dilators such as adenosine or dibutyl-cGMP in rat renal arteries (1), suggesting that the inhibitory effects of this compound on renal vasodilator responses are specific to NO-dependent dilators. We also examined the effect of preventing the fall in 20-HETE levels on the vasodilator response to SNP by adding exogenous 20-HETE to the bath. This maneuver also greatly attenuated the vasodilator actions of SNP. Together, these results suggest that inhibition of the formation of 20-HETE plays a major role in the renal vasodilator response to NO.

We also examined whether the effects of blocking the cGMP and the P-450 pathways were additive. Blocking both pathways completely eliminated the vasodilator response to SNP in renal vessels. Together, these results suggest that the vasodilator effects of NO can be completely explained on the basis of its effects on cGMP and 20-HETE formation and that one does not have to invoke a contribution of the potential direct effects of NO on K_{Ca} channels to explain the vasodilator response.
duced a dilation that averaged ~70% of the dilation produced by SNP, indicating that blockade of 20-HETE production in renal vessels without an intact endothelium exhibits similar dilator effects to those of NO. Thus it appears that P-450 inhibitors may not have a net effect on baseline diameter of intact renal arteries, because they block both the formation of a vasoconstrictor in VSM cells and the formation of vasodilators in the endothelium. It is also possible that P-450 inhibitors do not mimic the dilator response of SNP in intact renal arteries, because NO is normally released from the endothelium of these vessels and the baseline release of NO would inhibit 20-HETE formation in VSM cells. If this is the case, then P-450 inhibitors would not have a major dilator effect because basal 20-HETE levels would be low in vessels with an intact endothelium. Removal of the endothelium, on the other hand, would remove NO production, and it would allow 20-HETE levels to rise in VSM cells. Thus, in denuded renal vessels, P-450 inhibitors would be allowed to block a higher level of basal 20-HETE production and show a vasodilator effect.

In summary, the results of the present study indicate that NO donors inhibit the production of 20-HETE by microsomes prepared from renal arteries. Blockade of the cGMP pathway in renal microvessels only attenuates the vasodilator response to SNP by ~20–30%. However, inhibiting of the endogenous production of 20-HETE or preventing the fall in 20-HETE levels markedly impairs the vasodilator response to SNP. These findings suggest that the vasodilator actions of NO in the renal microcirculation are largely cGMP independent and that inhibition of the endogenous 20-HETE production contributes to the cGMP-independent dilator actions of NO in the renal circulation.

We thank Lisa Henderson for excellent technical assistance with the reverse-phase HPLC analysis of P-450 metabolites. This work was supported by National Institutes of Health Grants HL-29587, HL-36279, and GM-31278. M. Alonso-Galicia was supported by a research fellowship award from the National Kidney Foundation.

Address for reprint requests: R. J. Roman, Dept. of Physiology, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI 53226–0509.

Received 5 December 1997; accepted in final form 27 May 1998.

REFERENCES


