Inhibition of 20-HETE Production Contributes to the Vascular Responses to Nitric Oxide

Magdalena Alonso-Galicia, Heather A Drummond, K Kishta Reddy, John R Falck, Richard J Roman

Abstract
Nitric oxide (NO) inhibits a variety of heme-containing enzymes, including NO synthase and cytochrome P450A1 and P2B1. The present study examined whether NO inhibits the production of 20-hydroxyeicosatetraenoic acid (20-HETE) by cytochrome P450A4 enzymes and whether blockade of the production of this substance contributes to the vascular effects of NO. Sodium nitroprusside (SNP, 10⁻³, 10⁻⁴, and 10⁻⁵ mol/L) reduced the production of 20-HETE by renal microsomes incubated with arachidonic acid to 82±4%, 29±4%, and 4±2% of control, respectively (n=5). Similar results were obtained with the use of 1-propanamine, 3-(2-hydroxy-2-nitroso-1-propylhydrazono) (n=3).

To determine whether inhibition of 20-HETE contributes to the vasodilatory effects of NO, the effects of dibromo-dodecenylmethylsulfinide (DDMS), a selective inhibitor of the formation of 20-HETE, on the response to SNP (10⁻⁷ to 10⁻³ mol/L) were examined in rat renal arterioles precontracted with phenylephrine (n=5). SNP increased vascular diameter in a concentration-dependent manner to 82±4% of control. After DDMS (25 µmol/L), SNP (10⁻¹ mol/L) increased vascular diameter by only 17±3%. The effects of DDMS on the mean arterial pressure (MAP) and renal blood flow (RBF) responses to infusion of an NO donor and a synthase inhibitor were also examined in thebutobarbital-anesthetized Sprague-Dawley rats. Infusion of MAHMA NONOate at 1, 3, 5, and 10 nmol/min reduced MAP by 16±2, 30±3, 40±5, and 48±5 mm Hg and lowered renal vascular resistance (RVR) by 15±3%, 26±2%, 30±3%, and 34±4% of control. After DDMS (10 mg/kg, n=7 rats), the MAP and RVR responses to L-hexamethylenediuronium (L-NH₂) averaged only 20% of those seen during control.

In other experiments, MAP increased by 32±4% and RBF fell to 56±3% of control after administration of N-nitro-L-arginine (L-NArg) (10 mg/kg IV). After DDMS (10 mg/kg, n=7 rats), MAP increased by only 19±4% and RBF fell by only 7±4% after L-NArg. These results indicate that NO inhibits cytochrome P450A4 enzymes and that inhibition of the production of 20-HETE contributes to the vasodilatory effects of NO.

Key Words
- nitric oxide
- vasculature
- enzymes

Recent studies have indicated that the effects of many renal vasodilators are dependent on the release of NO from the endothelium. Blockade of NO synthesis increases arterial pressure, decreases RBF, and potentiates tubuloglomerular feedback responses. These results indicate that tone release of NO plays an important modulatory role in the regulation of both renal and peripheral vascular tone. It is generally assumed that the vasodilatory effects of NO are mediated by cGMP secondary to stimulation of guanylyl cyclase. This conclusion is based on the observations that endothelium-dependent vasodilators and NO donors increase cGMP in vascular tissue and that methylene blue and other inhibitors of guanylyl cyclase in many vessels can eliminate the vasodilatory response. However, this generalized scheme for NO-induced vasodilation has been questioned recently because there are an increasing number of reports that NO donors and/or endothelium-dependent dilators can produce vasodilatation and/or membrane hyperpolarization in some vessels in the presence of guanylyl cyclase inhibitors.

In the renal microcirculation, there have been no direct pharmacological or biochemical studies to examine the mechanisms by which NO or endothelium-dependent dilators alter renal vascular tone. It is not known whether the renal vasodilatory response to NO is dependent on the generation of cGMP or if a "cGMP-independent" signaling pathway also contributes to this response. In this regard, it should be noted that NO has recently been reported to inhibit NO synthase and P450 enzymes of the P450A family and whether blockade of the production of 20-HETE contributes to the effects of NO on renal vascular tone, both in vivo and in vitro.

Methods

Experiments were performed on 10- to 12-week-old male Sprague-Dawley rats purchased from Harlan Sprague Dawley Laboratories (Indianapolis, Ind). The rats were housed in an animal care facility at the Medical College of Wisconsin, which is approved by the American Association for the Accreditation of Laboratory Animal Care, and had free access to food and water.
Selected Abbreviations and Acronyms

AA = arachidonic acid
DDMS = dibromo-dodecyl-methylsulfoximide
DiHETEs = dihydroxyeicosatetraenoic acids
EETs = eicosatrienoic acids
20-HETE = 20-hydroxyeicosatetraenoic acids
HPLC = high-performance liquid chromatography
L-NA = N-nitro-L-arginine
MAHMA NONOate = 1-propylamine, 3-(2-hydroxy-2-nitroso-1-propylhydroxazine)
PAPA NONOate = 1-propanamine, 3-(2-hydroxy-2-nitroso-1-propylhydroxazine)
RBF = renal blood flow
SNP = sodium nitroprusside

All protocols involving animals received approval by the Animal Care Committee of the Medical College of Wisconsin

Renal Metabolism of AA

Adult male rats were anesthetized with an intraperitoneal injection of pentobarbital (50 mg/kg). The kidneys were removed and placed in ice-cold 0.9% NaCl solution. The renal cortex was homogenized in a 10 mmol/L potassium phosphate buffer (pH 7.4) containing 5 mmol/L potassium phosphate buffer (pH 7.4), 10 mmol/L magnesium chloride, and microsomes were prepared by differential centrifugation as we have previously described in the renal cortex microsomes (0.5 mg) for 30 minutes at 37°C with [1-14C] AA for 30 minutes in the presence of NADPH under control conditions and after addition of the various compounds to be tested. The reactions were terminated by acidification to pH 4 through the use of 0.1 mol/L formic acid and followed by extraction of the tissue and media with ethyl acetate. Metabolites were separated through the use of a 25 cm x 2 mm ID (Supelco Inc) C18-reverse-phase HPLC column and a linear elution gradient ranging from acetonitrile/water/acetate acid (50/50/0.1) to acetonitrile/acetic acid (100/0.1) over a 40-minute period. The radioactive products were monitored with the use of a radioactivity flow detector (model 120, Radiomatic Instrument Co).

Protocol 1: Effects of NO Donors on AA Metabolism

Renal cortical microsomes from male rats were incubated with [1-14C] AA for 30 minutes in the presence of NADPH under control conditions and after addition of various concentrations of SNP (10−5, 10−4, and 10−3 mol/L) or PAPA NONOate (10−4, 10−3, and 10−2 mol/L) (Cayman Chemical Co), a non-cyamde-releasing NO donor, to the incubation. The reactions were terminated by acidification to pH 4 through the use of 0.1 mol/L formic acid and followed by a lipid extraction of the tissue and media. Metabolites were then separated by reverse-phase HPLC as described above.

Protocol 2: Role of 20-HETE in the Response to NO Donors in Isolated, Perfused Renal Arterioles

Experiments were performed on isolated, perfused renal arterioles (<100 μm) microdissected from the kidneys of adult male rats. Rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.). The left kidney was removed and placed in ice-cold physiological saline solution (PSS) containing (in mmol/L) 119 NaCl, 4.7 KCl, 1.6 CaCl₂, 1.2 MgSO₄, 1.2 NaH₂PO₄, 12 NaHCO₃, 10 glucose, and 0.03 EDTA, pH 7.4. The kidney was hemidissected, and small interlobular arterioles with 50- to 100-μm ID were removed by microdissection. The vessels were mounted on glass micropipettes in a water-jacketed perfusion chamber containing PSS that was equilibrated with a 95% O₂-5% CO₂ gas mixture and maintained at 37°C. Indomethacin (5 μmol/L, Sigma Chemical Co) and baecaline (0.5 μmol/L, Biomol) were added to the bath to block the endogenous metabolism of AA through the cyclooxygenase and lipooxygenase pathways, as we have previously described. Vessels were secured to the pipettes with 10-0 silk suture, and side branches were tied off. The micropipette was connected to a pressurized reservoir to allow for control of intraluminal perfusion pressure, which was monitored with the use of a transducer (Cobe). After mounting, the vessels were stretched to the in vivo length with the use of an eyepiece micrometer, the outflow cannula was clamped off, and intraluminal pressure was set to 40 mm Hg during the experiment.

After a 30-minute equilibration period, the vessels were preconstricted with phenylephrine (1 μmol/L, Sigma Chemical Co), and a cumulative concentration-response curve for SNP (10−10 to 10−3 mol/L, Sigma Chemical Co) was obtained. Vascular diameters were measured 1 minute after the addition of SNP in the bath with the use of a video system composed of a stereomicroscope (Carl Zeiss, Inc), a CCTV video camera (KP-130AU, Hitachi), a videocassette recorder (AG-7300, Panasonic), a video monitor (CVM-1271, Sony), and a video measuring system (VIA-100, Boeckeler Instrument Co). The bath solution was then exchanged for freshly prepared PSS containing 5 μmol/L indomethacin, 0.5 μmol/L baecaline, and 25 μmol/L DDMS (n=5 rats) or vehicle (n=5 rats). Thirty minutes after blockade of the production of 20-HETE by DDMS, an experimental cumulative concentration-response curve for SNP was obtained after again preconstricting the vessels with phenylephrine (1 μmol/L).

To determine whether the effects of DDMS were specific to NO donors and not caused by a generalized inhibitory action, we studied the effects of DDMS on the vasodilatory responses to the NO-independent dilators dibutyryl-cGMP and adenosine. In these studies, cumulative concentration-response curves for N²-O-dibutrylguanosine 3’5’-cyclic monophosphate (dibutyryl-cGMP) (Sigma Chemical Co, n=3 vessels, 3 rats) and adenosine (Sigma Chemical Co; n=3 vessels, 3 rats) were obtained in vessels preconstricted with phenylephrine under control conditions and after 30 minutes of blockade of the production of 20-HETE by DDMS (25 μmol/L).

Finally, cumulative concentration-response curves for SNP also were obtained in phenylephrine-preconstricted renal microvessels under control conditions and after addition of 100 nmol/L of 20-HETE to the bath to determine whether preventing the fall in 20-HETE levels would block the vasodilatory response to SNP (n=6 vessels, 5 rats).

Protocol 3: Influence of 20-HETE on the Renal and Peripheral Vascular Responses to NO Donors

Adult male rats were anesthetized with ketamine (30 mg/kg) and thopobarbital (Ineetin) (100 mg/kg) and maintained at 37°C. Cannulas were placed in a femoral artery and vein for the measurement of systemic blood pressure and intravenous infusions. The rats received an intravenous infusion of 0.9% NaCl solution containing 3% albumin at a rate of 1.2 mL/h throughout the experiment. After surgery and a 30-minute equilibration period, MAP and RBF (measured with an electromagnetic flowmeter) were recorded during a control period and in response to 2-minute intravenous infusion of the very short-acting NO donor MAHMA NONOate (Cayman Chemical Co) at doses of 1, 3, 5, and 10 nmol/min. This donor was selected because it does not accumulate in the systemic circulation and blood pressure and RBF rapidly return to control (within 15 seconds) after the infusion is stopped. Five minutes, however, was allowed between each dose to allow for full equilibration of the preparation before testing the effects of the next dose. After the control responses were recorded, a 2 mg IV bolus injection of DDMS (6 mg/kg) was given followed by a maintenance infusion at a rate of 1 2
Effects of DDMS on the Renal Metabolism of AA

The effects of various concentrations of DDMS on the metabolism of AA by rat renal cortical microsomes are presented in Fig 1. Under control conditions (Fig 1A), renal cortical microsomes produced 14-15-, 11,12-, and 8,9-DiHETEs (retention times, 7, 8, and 8.5 minutes, respectively) and 20-HETE (retention time, 10 minutes) when incubated with AA. Addition of DDMS (Fig 1B) selectively reduced the formation of 20-HETE. At concentrations of 10⁻⁶ and 10⁻⁵ mol/L, DDMS inhibited the endogenous production of 20-HETE by rat renal cortical microsomes to 81±4% and 18±9% of control values, respectively (n=3), but had very little effect on the formation of EETs and DiHETEs (Fig 1C).

Protocol 1: Effects of NO Donors on Renal Metabolism of AA

The results of these experiments are summarized in Fig 2. Under control conditions, rat renal cortical microsomes produced 20-HETE, 14,15-, 11,12-, and 8,9-DiHETEs when incubated with AA (Fig 2A). Addition of 10⁻³ mol/L SNP to the incubation media completely blocked the metabolism of AA by P450 enzymes (Fig 2B). The effects of SNP on the renal metabolism of AA were concentration dependent. At concentrations of 10⁻⁳, 10⁻⁴, and 10⁻⁵ mol/L, SNP reduced the production of 20-HETE to 71±5%, 29±4%, and 42±2% of control (Fig 2C) and DiHETEs to 80±4%, 54±3%, and 10±3% of control.

Results

Protocol 4: Influence of 20-HETE on the Renal and Peripheral Vascular Responses to L-NArg

Additional experiments were performed to test the effects of systemic administration of the 20-HETE inhibitor on the renal and peripheral vascular responses to MAHA NONOate were once again assessed.

Statistics

Values are presented as mean±SEM. The significance of the differences in mean values within and between groups was examined with the use of ANOVA for repeated measures followed by Duncan’s multiple range test. A value of P<0.05 with a two-tailed test was considered significant.

Fig 1 Effects of various concentrations of DDMS on the metabolism of AA by rat renal cortical microsomes. A and B, Representative reverse-phase HPLC of the metabolites produced from AA under control conditions and after the addition of 5 µM DDMS, respectively. C, Effects of DDMS on the formation of EETs, DiHETEs, and 20-HETE expressed as percent of control. Values are mean±SEM obtained from renal cortical microsomes prepared from 3 rats.

Fig 2 Effects of various concentrations of two different NO donors, SNP and PAPA NONOate, on the metabolism of AA by rat renal cortical microsomes. A and B, Representative reverse-phase HPLC of the metabolites produced from AA under control conditions and after the addition of 5 µM DDMS to the incubation solution. Values are mean±SEM obtained from renal cortical microsomes prepared from 3 rats.
Under control conditions, SNP (10^{-7} to 10^{-4} mol/L, n=6 vessels) increased the inner diameter of these vessels by 64±4% of control. After fixing of vascular 20-HETE levels, the diameter of these vessels increased by only 24±4% of control. These results suggest that a fall in 20-HETE levels contributes significantly to the vasodilator response to NO donors.

To determine whether DDMS has any nonspecific inhibitory effects, the effects of this drug on the renal vasodilator responses to the NO-independent dilators adenosine and dibutyryl-cGMP were also examined. Under control conditions, adenosine (10^{-7} to 5×10^{-4} mol/L, n=3 vessels, 3 rats) increased the inner diameter of vessels preconstricted with phenylephrine by 68±10% of control. After blockade of the synthesis of 20-HETE with DDMS, adenosine increased vascular diameter by 79±9% of control. Similarly, dibutyryl-cGMP (10^{-7} to 10^{-4} mol/L, n=3 vessels, 3 rats) increased the diameter of vessels preconstricted with phenylephrine by 69±6% of control and by 61±10% of control after the synthesis of 20-HETE was blocked with DDMS.

Protocol 3: Role of 20-HETE in the Renal and Peripheral Vascular Responses to NO Donors

These experiments examined the effects of systemic administration of DDMS on the renal and peripheral vascular responses to NO donors in the rat in vivo. Control MAP averaged 115±5 mm Hg, RBF was 6.7±1 mL/min per gram of kidney weight, and RVR averaged 176±3 mL·min^{-1}·g^{-1}·mm Hg^{-1} (n=7 rats). Under these conditions, intravenous infusion of MAHMA NONOate at doses of 1, 3, 5, and 10 nmol/min reduced MAP to 99±3, 85±3, 75±6, and 68±6 mm Hg, respectively (Fig 4, top), and lowered RVR to 15±3%, 26±2%, 30±3%, and 34±4% of control values, respectively (Fig 4, bottom). After administration of DDMS (10 mg/kg), baseline MAP fell to 103±6 mm Hg and RVR was lowered to 15.3±1.5 mL·min^{-1}·mm Hg^{-1}. After DDMS pretreatment,
Peripheral Vascular Responses to L-NArg

These cGMP-independent effects of NO remains to be
polarization of VSM in many vascular beds in the pres-
vasodilators can produce vasodilation and/or hyper-
m vascular smooth muscle (VSM) cells and the production
by cGMP secondary to the stimulation of guanylyl cyclase
of both renal and peripheral vascular tone. It is generally
NO plays an important modulatory role in the regulat-
6.320 7 to 5.0±0 4 mL/mm per gram of kidney weight
Similarly, RBF in the DDMS-treated rats fell only from
104±4 to 122±6 mm Hg after administration of L-NArg
Thus, it appears that DDMS is a first-generation,
selective inhibitor of 20-HETE production, with an
=10-fold selectivity to inhibit renal ω-hydroxylation over epoxygenase activity.

In the present study, administration of DDMS at a con-
centrations of 60 μmol/L, selectively inhibited the
formation of NO donors in vessels treated with DDMS or
20-HETE is an endogenously formed constrictor of preglomerular
renal and cerebral arteries that normally inhibits the
opening of the large-conductance Ca²⁺-activated K⁺ channels in
VM, etc. 21,22 Inactivation of this channel results in
membrane depolarization and promotes vasoconstriction by
increasing calcium influx through voltage-sensitive
Ca²⁺ channels. When NO binds to P450 enzymes in
VM, it inhibits the endogenous production of 20-HETE.
The subsequent fall in 20-HETE levels leads to activation of
the large-conductance Ca²⁺-activated K⁺ channels and
membrane hyperpolarization. Hyperpolarization of renal
VM cells would be expected to decrease calcium influx
through voltage-sensitive Ca²⁺ channels and promote vaso-
sodilation. When the endogenous synthesis of 20-HETE is
blocked with DDMS, or intracellular levels of 20-HETE
are fixed by exogenous addition of the compound, NO
cannot lower 20-HETE levels and promote vasoconstriction by
hyperpolarizing the membrane through activation of
Ca²⁺-activated K⁺ channels. Thus, the response to NO
donors is attenuated by 70%. The residual vasodilatory re-
response to NO donors in vessels treated with DDMS or
20-HETE may represent the cGMP-dependent component of
the vasodilatory response to NO. It is important to note
that the vasodilatory response to NO-independent dilators
adenosine and dibutyryl-cGMP in renal vessels was not
significantly altered by DDMS. These results suggest that
the inhibitory effects of DDMS on renal vasodilatory re-
sponses are specific to NO-dependent dilators.

Experiments were also performed to determine the possible
role of 20-HETE in the renal and peripheral vasodi-
latory effects of NO donors and synthesize inhibitors in rats in vivo. In these experiments, the dose of DDMS was
chosen on the basis of preliminary experiments indicating that
infusion of DDMS (10 mg/kg) over a 1-hour period (either
into the renal artery or intravenously) produced the same

Discussion
A large number of studies suggest that tonic release of
NO plays an important modulatory role in the regulation of
both renal and peripheral vascular tone. It is generally
believed that the vasodilatory effects of NO are mediated
by cGMP secondary to the stimulation of guanylyl cyclase
in vascular smooth muscle (VSM) cells and the production
cGMP. However, an increasing number of studies have
indicated that NO donors or endothelium-dependent
vasodilators can produce vasodilation and/or hyper-
polarization of VSM in many vascular beds in the pres-
ence of guanylyl cyclase inhibitors. The mechanism of
these cGMP-independent effects of NO remains to be
determined.

In the present study we explored the possibility that NO
may inhibit the formation of 20-HETE and that this may
contribute to the vasodilatory effects of NO. This hypo-
thesis is based on the recent observations that NO inhibits
NO synthase and P450 enzymes of the 1A and 2B19 and
3C10 families and from studies indicating that renal VSM
cells metabolize AA through a P4504A-dependent path-
way to 20-HETE13,14 and that this system plays a central
role in the regulation of renal vascular tone.15-17 The present
finding that two different NO donors (SNP and PAPA
NOnoate) blocked the formation of 20-HETE in a con-
centration-dependent manner in rat renal cortical microsomes
provides direct evidence to support this possibility.

To study the role that inhibition of the endogenous
production of 20-HETE plays in the vasodilatory re-
sonse to NO, we had to find a way to selectively block
the system both in vivo and in vitro. In previous studies we
have reported that miconazole is a highly selective
inhibitor of renal epoxygenase activity but has little effect
on the formation of 20-HETE.20 17-ODYA, on the other
hand, inhibits the formation of both EET and 20-HETE
in the kidney in vivo when administered directly into the
renal interstitium; however, it is extensively protein
bound and does not inhibit renal P450 activity when
given intravenously.20

In the present study we evaluated the potential usefulness of a new compound (DDMS) that was synthesized by
Dr. Falck to be a selective, mechanism-based inhibitor of
P450 enzymes of the 4A family that catalyze ω-hydroxy-
laric acid analogue, which is the preferred substrate for
the P4504A enzymes, with a dibromo modification on the
terminal carbon designed to bind to the active site of the
enzyme. In addition, the molecule has a methyl sulfimide
modification on the C1 carbon to enhance its solubility in
aqueous solutions and to increase the half-life by blocking
β-oxidation of the molecule. The effects of DDMS on the
metabolism of AA in the kidney were evaluated by incuba-
ting renal cortical microsomes with [14C]-AA in the
presence of NADPH. We demonstrated that DDMS at a
concentration of 10 μmol/L, selectively inhibited the
ω-hydroxylation of AA to 20-HETE by rat renal cortical
microsomes, but it had only a slight effect on the formation
of DiHETEs4.6 At higher concentrations (100 μmol/L), how-
ever, DDMS, like 17-ODYA, did inhibit the formation of
EETs and DiHETEs by renal cortical microsomes (data not shown). These observations support the hypothesis that inhibition of the
formation of 20-HETE in renal arterioles contributes to the
vasodilatory response to NO, however, the mechanism of
this interaction remains to be established.

In previous studies we have reported that 20-HETE is
an endogenously formed constrictor of preglomerular
renal and cerebral arteries that normally inhibits the
opening of the large-conductance Ca²⁺-activated K⁺ channels in
VM. 21,22 Inactivation of this channel results in
membrane depolarization and promotes vasoconstriction
by increasing calcium influx through voltage-sensitive
Ca²⁺ channels. When NO binds to P450 enzymes in
VM, it inhibits the endogenous production of 20-HETE.
The subsequent fall in 20-HETE levels leads to activation of
the large-conductance Ca²⁺-activated K⁺ channels and
membrane hyperpolarization. Hyperpolarization of renal
VM cells would be expected to decrease calcium influx
through voltage-sensitive Ca²⁺ channels and promote vaso-
sodilation. When the endogenous synthesis of 20-HETE is
blocked with DDMS, or intracellular levels of 20-HETE
are fixed by exogenous addition of the compound, NO
cannot lower 20-HETE levels and promote vasoconstriction by
hyperpolarizing the membrane through activation of
Ca²⁺-activated K⁺ channels. Thus, the response to NO
donors is attenuated by 70%. The residual vasodilatory re-
response to NO donors in vessels treated with DDMS or
20-HETE may represent the cGMP-dependent component of
the vasodilatory response to NO. It is important to note
that the vasodilatory response to NO-independent dilators
adenosine and dibutyryl-cGMP in renal vessels was not
significantly altered by DDMS. These results suggest that
the inhibitory effects of DDMS on renal vasodilatory re-
sponses are specific to NO-dependent dilators.

Experiments were also performed to determine the possible
role of 20-HETE in the renal and peripheral vasodi-
latory effects of NO donors and synthesize inhibitors in rats in vivo. In these experiments, the dose of DDMS was
chosen on the basis of preliminary experiments indicating that
infusion of DDMS (10 mg/kg) over a 1-hour period (either
into the renal artery or intravenously) produced the same
degree of renal vasodilation and blockade of the autoregulation of RBF as a dose of 17-ODYA, which reduces renal 20-HETE production by 70%. In addition, Brand-Schleiber et al. have recently reported that a similar dose of DDMS effectively reduces the urinary excretion of 20-HETE, an index of renal 20-HETE production. We found that DDMS inhibited the renal vasodilatory response to graded infusions of an NO donor by ≈80%. It also inhibited the rise in renal vascular resistance produced by a maximal vasoconstrictor dose of an inhibitor of NO synthesis by ≈50%. These findings are consistent with the results of the in vitro studies on isolated renal arterioles and together suggest that inhibition of the production of 20-HETE may contribute to the vasodilatory effects of NO in the renal microcirculation.

It was also surprising to see that systemic administration of DDMS greatly attenuated the fall in blood pressure produced by intravenous infusion of MAHMA NONOate and DDMS greatly attenuated the fall in blood pressure produced by L-NArg.

With the results of the in vitro studies on isolated renal arterioles, it was also surprising that systemic administration of DDMS greatly attenuated the fall in systemic blood pressure produced by L-NArg. These observations suggest that NO may tonically inhibit the formation of 20-HETE in the rat in vivo in vascular beds other than the kidney and the brain, in which we have established a prominent role for this substance in the regulation of vascular tone.

Clearly, more work is needed to map the vascular beds that produce 20-HETE and the relative contribution of 20-HETE and NO to the regulation of basal vascular tone.

In summary, the present results suggest that NO inhibits renal cytochrome P450A enzymes and that inhibition of the endogenous production of 20-HETE contributes to the effects of NO on renal and peripheral vascular tone.

Acknowledgments

This work was supported by grants from the National Institutes of Health HL-29587, HL-36279, and GM 31278. The authors wish to thank Lisa Henderson for excellent technical assistance with the P450 assays.

References

2. Loscalzo J, Welch G. Nitric oxide and its role in the cardiovascular system. Prog Cardiovasc Dis 1995,38 87-104
4. Cohen RA. The role of nitric oxide and other endothelial derived vasoactive substances in vascular disease. Prog Cardiovasc Dis 1995,38 105-128
10. Kluesenego OG, Gross SS, Rforkn AB, Vase JR. Nitric oxide is a mediator of the decrease in cytochrome P450-dependent metabolism caused by immunostimulants. Proc Natl Acad Sci USA 1993,90 11147-11151
12. Schertz HS, Duthu GS. Nitrate binding to rabbit blood microsomes and effects of aminopyridine demethylation. Biochem Pharmacol 1979,28 873-879

Abstract
Inhibition of 20-HETE Production Contributes to the Vascular Responses to Nitric Oxide
Magdalena Alonso-Galicia, Heather A. Drummond, K. Kishta Reddy, John R. Falck and Richard J. Roman

Hypertension. 1997;29:320-325
doi: 10.1161/01.HYP.29.1.320

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1997 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/29/1/320

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org/subscriptions/