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

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

Abstract
Nitric oxide (NO) inhibits a variety of heme-containing enzymes, including NO synthase and cytochrome P450A enzymes and whether blockade of the production of NO contributes to the vasodilatory effects of NO. Sodium nitroprusside (SNP, 10^{-3}, 10^{-2}, and 10^{-1} mol/L) reduced the production of 20-HETE by renal microsomes incubated with arachidonic acid to 71±5%, 26±2%, and 1±2% of control, respectively (n=5). Similar results were obtained with the use of 1-propanamine, 3-(2-hydroxy-2-nitroso-1-propylhydrazine) (n=3). To determine whether inhibition of 20-HETE contributes to the vasodilatory effects of NO, the effects of dibromo-dodecenylmethylsulfoxide (DDMS), a selective inhibitor of the formation of 20-HETE, on the response to SNP (10^{-1} to 10^{-3} mol/L) were examined in rat renal arterioles preconstricted 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 synthetic inhibitor were also examined in thioacetamidem-asthesitized Sprague-Dawley rats. Infusion of MAHMA NONOate at 1, 3, 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 1-hexamene, 6-(2-hydroxy-1-methyl-2-nitrohydrindazino)N-methyl (MAHMA NONOate) 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 P450A enzymes and that inhibition of the production of 20-HETE contributes to the vasodilatory effects of NO (Hypertension. 1997;29[part 2]:320-325.)

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.1 These results indicate that tonic 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 cyclase2-3. 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 generalization 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 vasodilation and/or membrane hyperpolarization in some vessels in the presence of guanylyl cyclase inhibitors.4-6

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.1 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 synthase7,8 and P450 enzymes of the 1A and 2B19 and 3C10 families by forming iron-nitrosyl complexes at the catalytic heme binding site in these enzymes. There is also a large body of evidence attributing the toxic effects of nitrates on drug metabolism in the liver to the formation of NO from nitrates and the subsequent inhibition of P450 enzymes.11,12 In light of the recent studies from our laboratory indicating that renal vascular smooth muscle cells metabolize AA through a P450A-dependent pathway, it is likely that this system plays a central role in the regulation of renal vascular tone,13,14 and that this system plays a central role in the regulation of renal vascular tone,15,16 it seems likely that NO might inhibit the formation of 20-HETE in renal vascular smooth muscle and that this might contribute to the vasodilatory effects of NO. Therefore, the purpose of the present study was to determine whether NO inhibits 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.

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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 250 mmol/L sucrose, 1 mmol/L EDTA, and 10 mmol/L magnesium chloride, and microsomes were prepared by differential centrifugation as we have previously described. The microsomes (0.5 mg) for 30 minutes at 37°C with [1-14C] AA for 30 minutes in the presence of NADPH under 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 homogenized and small interlobular arterioles with 50-100 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 balsalicylic acid (0.5 µmol/L, Sigma Chemical Co) were added to the bath to block the endogenous metabolism of AA through the cyclooxygenase and lipoxygenase pathways, as we have previously described. Vessels were secured to the pipettes with 10-0 silk suture, and side branches were tied off. The intraluminal pressure was monitored with a pressure transducer (Statham) 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 using an eye piece micrometer, and the outflow cannula was clamped off, and intraluminal pressure was set to 30 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 (10-5 to 10-2 mol/L, Sigma Chemical Co) was obtained. The cumulative concentration-response curves for SNP were obtained 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 (10-5 to 10-2 mol/L, Sigma Chemical Co) was obtained. The cumulative concentration-response curves for SNP were obtained 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 (10-5 to 10-2 mol/L, Sigma Chemical Co). The vascular diameters were measured 1 minute after the addition of SNP to 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), and a video monitor (CVM-1271, Sony), and a video monitoring 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 balsalicylic acid, and 25 µmol/L DDMS (n=6 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'-dibutyrylguanosine 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 µmol/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 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 (10-5, 10-4, and 10-3 mol/L) (Cayman Chemical Co), a non-cyanide-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 liquid extraction of the tissue and media with ethyl acetate. Metabolites were separated through the use of a 25 cm×2 mm ID (Supelco Inc) C18-reverse-phase HPLC column 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-minute period. The radioactive products were monitored with the use of a radioactive flow detector (model 120, Radiomatic Instrument Co).

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 ketamine (30 mg/kg) and thiobutabarbital (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 MAHMMA 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

Selected Abbreviations and Acronyms

AA = arachidonic acid
DDMS = dibromo-dodecyl-methylsulfoxide
DiHETEs = dihydroxyeicosatetraenoic acids
EETs = eicosatetraenoic acids
20-HETE = 20-hydroxyeicosatetraenoic acids
HPLC = high-performance liquid chromatography
L-NArg = L-arginine-1-arginine
MAHMA NONoate = 1-hexamine, 6-(2-hydroxy-1-methyl-
and media with ethyl acetate Metabolites were separated through the use of a radioactive flow detector (model 120, Radiomatic Instrument Co).

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 thiobutabarbital (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 MAHMMA 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

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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. Renal microsomes produce 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. Values are mean ± SEM obtained from renal cortical microsomes prepared from 3 rats.

mg/h. After a 1-hour equilibration period, the effects of systemic administration of the 20-HETE inhibitor on the renal and peripheral vascular responses to MAHMA NONOate were once again assessed.

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 a 20-HETE inhibitor on the changes in arterial pressure and RBF produced by a maximal dose of the NO synthase inhibitor L-NArg (10 mg/kg IV, Sigma Chemical Co). In these experiments, rats were surgically prepared as described above, and after a 30-minute control period, baseline RBF and MAP were measured. One group of rats (n=7) received the 20-HETE inhibitor DDMS as described in Protocol 3, while the other group (n=7) received only vehicle (saline containing 20 mmol/L Na2CO3). One hour later, RBF and MAP were again measured, and the rats were given an intravenous bolus injection of L-NArg (10 mg/kg). After 15 minutes, RBF and MAP were again recorded.

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.

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 it 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 10⁻³, 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 100±4%, 54±3%, and 10±3% of control.

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 μmol/L DDMS to the incubation solution C. Effects of DDMS on the formation of EETs, DiHETEs, and 20-HETE expressed as percent of control. Retention times are described in Fig 1. Values are mean ± SEM obtained from renal cortical microsomes prepared from 3 rats.
Under control conditions, SNP (10⁻⁷ to 10⁻³ mol/L, n=6) vent the fall in vascular 20-HETE levels produced by SNP concentration of 20-HETE was added to the bath to pre-
fall in 20-HETE levels that contributes to the vasodilator HETE and D₁HETEs, we also examined whether It is the
mstratlon of vehicle to the bath were not significantly
for SNP under control condltlons and 30 minutes after ad-
tachyphylaxls or to a time-dependent fall m the respon-
siveness of the preparation, time control expenments were
performed In five vessels, concentration-response curves
of SNP. To rule out the posslbhty that the vasodilatory
response to SNP in the presence of DDMS was due to
tachyphylaxis or to a time-dependent fall m the respon-
percent of dllatlon Values are mean±SEM obtained from vessels
from 5 rats *Significant difference from control values

respectively. Similar results were obtained with the use of a non–cyamde-releasing NO donor (PAPA NONOate) At
concentrations of 10⁻⁵, 10⁻⁴, and 10⁻³ mol/L. PAPA NONOate reduced the production of 20-HETE to 87±3%,
30±13%, and 2±2% of control and D₁HETEs to 93±3%,
22±2%, and 2±2% of control, respectively The effects of MAHMA NONOate on the renal metabolism of AA were
not studied because It is a short-acting donor and cannot produce the sustained release of NO that is needed for
these incubation experiments

Protocol 2: Influence of 20-HETE on the Vasodilatory Response to SNP in Isolated, Perfused Renal Arterioles

The contribution of 20-HETE to the vasodilatory re-
response to an NO donor was determined by comparison of
the concentration-response relation to SNP on vascular di-
ater before and after blocking of the endogenous pro-
duction of 20-HETE with DDMS (25 µmol/L) These re-
results are summarized in Fig 3 The control inner diameter
of the vessels was 98±10 µm (n=5 vessels, 5 rats). Phenylephrine (1 µmol/L) reduced the diameter of these vessels by
≈50% to 45±3 µm SNP (10⁻⁷ to 10⁻³ mol/L) increased the diameter of these vessels in a concentra-
dependent manner to a maximum of 82±4% of control. After
DDMS, the vasodilatory response to SNP was greatly attenuated. Vessel diameters increased to only
17±3% of control in response to the highest concentra-
of SNP. To rule out the possibility that the vasodilatory
response to SNP in the presence of DDMS was due to
tachyphylaxis or to a time-dependent fall in the respon-
siveness of the preparation, time control experiments were
performed In five vessels, concentration-response curves
for SNP under control conditions and 30 minutes after ad-
mistration of vehicle to the bath were not significantly
different (data not shown).

Because NO donors inhibit the formation of both 20-
HETE and D₁HETEs, we also examined whether it is the
fall in 20-HETE levels that contributes to the vasodilator
response to NO donors. In these experiments, a fixed high
concentration of 20-HETE was added to the bath to pre-
vent the fall in vascular 20-HETE levels produced by SNP. Under control conditions, SNP (10⁻⁷ to 10⁻³ mol/L, n=6
vessels, 5 rats) increased the inner diameter of these ves-
sels 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 vaso-
dilator response to NO donors.

To determine whether DDMS has any nonspecific inhibitory effects, the effects of this drug on the renal va-
sodilatory responses to the NO-independent dilators aden-
osine and dbutyrly-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 pre-
constricted with phenylephrine by 68±10% of control. Af-
ter blockade of the synthesis of 20-HETE with DDMS, adenosine increased vascular diameter by 79±9% of control.
Similarly, dbutyrly-cGMP (10⁻⁷ to 10⁻⁴ mol/L, n=3
vessels, 3 rats) increased the diameter of vessels precon-
stricted 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 ad-
ministration 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 17 6±1 3
mL⁻¹ min⁻¹ g⁻¹ mm Hg⁻¹ (n=7 rats). Under these condi-
tions, intravenous intusion 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⁻¹.min⁻¹ g⁻¹ mm Hg⁻¹. After DDMS pretreatment,

![Fig 3](image-url) Cumulative concentration-response curves for the effects of SNP on the inner diameters of isolated, perfused rat renal arterioles preconstricted with phenylephrine (1 µmol/L). Vessels (n=5) were studied under control conditions and after the addition of 25 µmol/L DDMS to the bath. Results are expressed as percent of dilation. Values are mean±SEM obtained from vessels from 5 rats *Significant difference from control values.

![Fig 4](image-url) Cumulative concentration-response curves for the in vivo effects of MAHMA NONOate on the MAP and renal vascular resistance of inactln-anesthetized rats. Animals were studied under control conditions and after intravenous administration of DDMS 10 mg/kg over a 1-hour period. Results are expressed as percent of control. Values are mean±SEM obtained from 7 rats *Significant difference from vehicle values.
Peripheral Vascular Responses to L-NArg

NO synthase and P450 enzymes of the 1A and 2B19 and these cGMP-independent effects of NO remains to be polarization of VSM in many vascular beds in the pres-

vaskodilators can produce vasodilation and/or hyper-

have indicated that NO donors or endothelium-dependent vascular smooth muscle (VSM) cells and the production by cGMP secondary to the strmulatron of guanylyl cyclase

believed that the vasodilatory effects of NO are mediated of both renal and peripheral vascular tone It is generally

the system both in vrvo and in vitro In previous studies we have reported that miconazole 1s a highly selectrve

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production of 20-HETE plays m the vasodilatory re-

somes provides direct evidence to support this possibthty

at concentrations of 10 μmol/L, selectively inhibited the 

enriched with guanylyl cyclase inhibitors.4,6 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 hypothesis 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 concentration-dependent manner in rats cortical microsome 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-

sponse 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 EETs 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 intravascularly.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-

lation of medium and long-chain fatty acids DDMS is a lauric 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 oxidation of the molecule. The effects of DDMS on the metabolism of AA in the kidney were evaluated by incubating 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 EETs and DiHETEs. At higher concentrations (100 μmol/L), however, DDMS, like 17-ODYA, did inhibit the formation of EETs and DiHETEs by renal cortical microsomes (data not shown) Thus, it appears that DDMS is a first-gener-

ation, 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-

centration similar to that which inhibited the formation of 20-HETE by renal microsomes in vitro greatly attenuated the vasodilatory response to SNP in renal arterioles pre-

constricted with phenylephrine We have also observed similar effects using 17-ODYA (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 re-

nal and cerebral arteries13,14 that normally inhibits the opening of the large-conductance Ca2+-activated K+ channels in VSM21,22 Inactivation of this channel results in membrane depolarization and promotes vasoconstriction by increasing calcium influx through voltage-sensitive Ca2+ channels When NO binds to P4504A enzymes in VSM, it inhibits the endogenous production of 20-HETE. The subsequent fall in 20-HETE levels leads to activation of the large-conductance Ca2+-actuated K+ channels and membrane hyperpolarization. Hyperpolarization of renal VSM cells would be expected to decrease calcium influx through voltage-sensitive Ca2+ channels and promote vasodilation. 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 vasodilation by hyperpolarizing the membrane through activation of Ca2+-actuated K+ channels. Thus, the response to NO don-

ors is attenuated by 70% The residual vasodilatory re-

sponse 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 responses 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 syntheh inhibitors in rats in vivo In these experiments, the dose of DDMS was cho-

sen 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,20 which reduces renal 20-HETE production by 70%. In addition, Brand-Schieber et al21 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 synthase 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 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.

In summary, the present results suggest that NO inhibits renal cortical 4504A enzymes and that inhibition of the endogenous production of 20-HETE contributes to the effects of NO on renal and peripheral vascular tone.

Acknowledgments

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