Role of guanylyl cyclase and cytochrome P-450 on renal response to nitric oxide

BERNARDO LÓPEZ,1 CAROL MORENO,1 MIGUEL GARCÍA SALOM,1 RICHARD J. ROMAN,2 AND FRANCISCO J. FENOY1
1Departamento de Fisiología, Facultad de Medicina, 30100-Murcia, Spain; and
2Department of Physiology, Medical College of Wisconsin, Milwaukee, Wisconsin 53226

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López, Bernardo, Carol Moreno, Miguel García Salom, Richard J. Roman, and Francisco J. Fenoy. Role of guanylyl cyclase and cytochrome P-450 on renal response to nitric oxide. Am J Physiol Renal Physiol 281: F420–F427, 2001.—The present study evaluated whether inhibition of guanylyl cyclase (GC) with 1H-(1,2,4)oxadiazolo[4,3-a]quinoxaline-1-one (ODQ) and methylene blue (MB) or inhibition of the renal metabolism of arachidonic acid by cytochrome P-450 (CYP450) enzymes with 1-aminobenzotriazole (ABT) alters the renal tubular and vascular effects of a nitric oxide (NO) donor in vivo. Intrarenal infusion of ODQ or MB at a dose of 170 nmol·kg⁻¹·min⁻¹ lowered renal blood flow (RBF) by 30 and 15%, respectively; glomerular filtration rate (GFR) by 26 and 18%, respectively; and sodium and water excretion by ~35%. In rats pretreated with nitro-l-arginine methyl ester (37 nmol·kg⁻¹·min⁻¹) to block the endogenous production of NO, intrarenal infusion of the NO donor S-nitroso-N-acetylcysteine (S-NO-NAC; 50 nmol·kg⁻¹·min⁻¹) increased RBF (18%), sodium (73%), and water excretion (61%). ODQ or MB administration blocked the effect of S-NO-NAC on RBF but not the diuretic and natriuretic response. Pretreatment of rats with ABT or HET0016 also abolished the renal vasodilatory response to the NO donor and reduced its diuretic and natriuretic effect. These results indicate that both activation of GC and inhibition of CYP450 enzymes contribute to the renal vascular actions of NO, whereas the natriuretic and diuretic actions of NO appear to be largely CYP450 dependent.

Recent studies have indicated that nitric oxide (NO) plays a central role in the regulation of renal tubular and vascular function and in the long-term control of arterial pressure (5, 6). Endothelial-derived NO affects vascular tone in both afferent and efferent arterioles (17). Despite the importance of NO in the control of renal function, its mechanism of action in the kidney is not well understood. It has generally been assumed that the renal actions of NO are solely mediated by activation of guanylyl cyclase (GC), which increases the levels of guanosine 3′,5′-cyclic monophosphate (cGMP). This conclusion is supported by the findings that endothelium-derived NO and NO donors increase cGMP levels in vascular tissue and that inhibitors of GC attenuate the vasodilatory response to NO in many vessels (14). Further support for this hypothesis is provided by the observation that the effects of Nω-nitro-l-arginine methyl ester (l-NAME), an inhibitor of nitric oxide synthase (NOS), on arterial pressure and renal function can be reversed by S-bromoguanosine 3′,5′-cyclic monophosphate (8-BrcGMP) (9). However, this scheme for NO-induced vasodilation recently has been questioned because in a variety of vascular beds, including the renal circulation, NO has been reported to act via both cGMP-dependent and cGMP-independent mechanisms (3, 29).

Recently, a new route for the metabolism of arachidonic acid (AA) has been described. This pathway, which is dependent on cytochrome P-450 (CYP450), NADPH, and molecular oxygen, produces a series of epoxyeicosatrienoic acids (EETs), dihydroxyeicosatrienoic acids (DiHETEs), and hydroxytetraenoic acids (HETEs) from AA. The primary metabolites formed in the kidney are 20-HETE; 14,15- and 11,12-EETs; and DiHETEs. The available data indicate that CYP450 metabolites of AA play a critical role in the regulation of both tubular and vascular function (15). CYP450 metabolites of AA participate in the myogenic response of renal arterioles to elevations in transmural pressure and in the regulation of renal vascular tone (7, 12). 20-HETE also inhibits Na⁺-K⁺-ATPase and sodium transport in the proximal tubule (21) and Na⁺-K⁺-2Cl⁻ transport in the thick ascending loop of Henle (4). Other studies have demonstrated that endogenous CYP450 metabolites of AA influence renal medullary hemodynamics and the excretion of water and electrolytes in vivo (31) and contribute to the long-term regulation of arterial pressure (26).

NO has been demonstrated recently to inhibit CYP450 enzymes of the 1A, 2B1, 3C, and 4A families by forming iron-nitrosyl complexes at the catalytic heme binding site in these enzymes (1, 18, 30). NO

Address for reprint requests and other correspondence: F. J. Fenoy, Departamento de Fisiología y Farmacología, Facultad de Medicina, Campus de Espinardo, 30100-Murcia, Spain (E-mail: ffenoy@um.es).

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donors also inhibit the synthesis of 20-HETE by renal tubular and vascular tissue (1, 2), and the inhibition of CYP450 enzymes attenuates the vasodilatory response of renal microvessels to NO in vitro to a much greater extent than GC inhibition (1, 2, 28). In addition, inhibitors of the formation of 20-HETE attenuate the systemic vasodilator response to NO donors (1) in rats in vivo. However, the relative contribution of GC and CYP450 metabolites of AA to the renal vascular and excretory effects of NO in vivo remains to be determined. Therefore, the present study examined the effects of the inhibition of GC with 1H-(1,2,4)oxadiazolo[4,3-a]quinoxaline-1-one (ODQ) or methylene blue (MB) and blockade of CYP450 metabolism of AA with 1-aminobenzotriazole (ABT) or N-hydroxy-N-[(4 butyl-2-methyl phenyl)formamidine (HET0016) on the renal hemodynamic and natriuretic actions of an intrarenal infusion of an NO donor in rats in vivo.

MATERIALS AND METHODS

Experiments were performed on 64 anesthetized male Sprague-Dawley rats (240–260 g) bred in the Animal Care Facility at the University of Murcia. All procedures performed were in accordance with the recommendations from the Declaration of Helsinki and the guiding principles in the care and use of animals approved by the American Physiological Society. The rats were anesthetized with an intramuscular injection of ketamine (30 mg/kg im, Rhône Merieux) and an intraperitoneal injection of thiopental (Pentothal, 50 mg/kg ip; Abbott). Cannulas were placed in the right femoral vein for infusions, in the right femoral artery for measurement of mean arterial pressure (MAP), and in the left ureter for collection of urine. A heat-pulled PE-10 catheter was inserted into the aorta and advanced into the left renal artery for intrarenal infusions (19). Isotonic NaCl solution was continuously infused at a rate of 50 μl/min to maintain the patency of the catheter. The left kidney was denervated, and an electromagnetic flow probe (Skalar) was placed around the left renal artery for measurement of renal blood flow (RBF). Plasma levels of vasopressin (0.17 ng·kg⁻¹·min⁻¹), aldosterone (66 ng·kg⁻¹·min⁻¹), and norepinephrine (333 ng·kg⁻¹·min⁻¹) were fixed at high physiological levels by an intravenous infusion of a hormone cocktail at a rate of 1 ml·100 g⁻¹·h⁻¹ (22). After surgery and a 90-min equilibration period, urine flow (UV), sodium excretion (U⁰Na⁰), RBF, glomerular filtration rate (GFR), and MAP were measured before and after intrarenal infusion of an NO donor, S-nitroso-N-acetyl-L-cysteine (S-NO-NAC) (25), in rats pretreated with vehicle or GC or CYP450 inhibitors. All drugs (except ABT and HET0016) were administered intravenously.

Group 1: time course studies. After two 15-min control periods, urine and plasma samples were collected at 30-min intervals for 2 h.

Group 2: renal effects of ODQ. Experiments were performed to find a dose of ODQ that alters renal function in vivo. UV, U⁰Na⁰, GFR, and RBF were measured during two 15-min control periods. Then, three different doses of ODQ (1.7, 17, and 170 nmol·kg⁻¹·min⁻¹) were infused into the renal artery to obtain concentrations in renal arterial plasma of ~0.1, 1, and 10 μmol/l. Each dose was infused for 30 min, and urine and plasma samples were again collected during three 15-min experimental clearance periods.

Additional experiments were performed to assess the degree of inhibition of GC after intrarenal infusion of the highest dose of ODQ. In these experiments, the right kidney was removed after surgery, quickly frozen in liquid nitrogen, and used as a control. ODQ (170 nmol·kg⁻¹·min⁻¹) was infused into the renal artery of the left kidney for 30 min, and then the kidney was quickly removed and frozen. The frozen kidneys were homogenized and centrifuged at 10,000 g, and the supernatants were incubated with 0.5 mmol/l isobutyl-methylxanthine and vehicle or 0.1 mmol/l S-NO-NAC at 37°C for 10 min to stimulate GC activity. The reactions were terminated by adding 5% trichloroacetic acid. The samples were centrifuged, and the supernatants were used for a commercially available enzyme immunoassay (EIA) (2), and the values were normalized per milligram of dry kidney weight.

Group 3: renal effects of MB. These experiments were performed to find a dose of MB that alters renal function in vivo. UV, U⁰Na⁰, GFR, and RBF were measured during two 15-min control periods. Then, different doses of MB (17, 170, and 1,700 nmol·kg⁻¹·min⁻¹) were infused into the renal artery to obtain concentrations in renal arterial plasma of ~1, 100, and 100 μmol/l. Each dose was infused for 30 min, and then urine and plasma samples were again collected during three 15-min experimental clearance periods.

Group 4: renal effects of L-NAME and the NO donor S-NO-NAC. These experiments were performed to determine the highest dose of the NO donor that modifies renal hemodynamics and U⁰Na⁰ without altering arterial pressure. After a basal clearance period, L-NAME (37 nmol·kg⁻¹·min⁻¹ for 30 min followed by 0.37 nmol·kg⁻¹·min⁻¹) was infused intrarenally to suppress the endogenous formation of NO. After a 30-min equilibration period, urine and plasma samples were collected during a 10-min clearance period. Then, the NO donor S-NO-NAC was infused intrarenally at doses of 0.5, 5, and 50 nmol·kg⁻¹·min⁻¹ for 15 min, and urine and plasma samples were again collected during three additional 15-min clearance periods.

Group 5: effects of ODQ on renal response to L-NAME and an NO donor. These experiments examined the effects of blocking GC activity with ODQ on the renal responses to an intrarenal infusion of the NO donor S-NO-NAC. These experiments were similar to those described in protocol 4 except that the rats received an intrarenal infusion of ODQ (170 nmol·kg⁻¹·min⁻¹) along with L-NAME (37 nmol·kg⁻¹·min⁻¹ for 30 min followed by 0.37 nmol·kg⁻¹·min⁻¹) throughout the experimental period. Then, S-NO-NAC at doses of 0.5, 5, and 50 nmol·kg⁻¹·min⁻¹ was sequentially infused intrarenally as described above.

Group 6: effects of MB on renal response to L-NAME and an NO donor. These experiments examined the effects of MB on the renal responses to intrarenal infusion of the NO donor S-NO-NAC. These experiments were similar to those described in protocol 5 except that the rats received an intrarenal infusion of MB (170 nmol·kg⁻¹·min⁻¹) and L-NAME (37 nmol·kg⁻¹·min⁻¹ for 30 min followed by 0.37 nmol·kg⁻¹·min⁻¹) throughout the experimental period. Then, S-NO-NAC at doses of 0.5, 5, and 50 nmol·kg⁻¹·min⁻¹ was infused intrarenally as described in protocol 4.

Group 7: renal effects of L-NAME and an NO donor in rats pretreated with ABT. These rats were pretreated with an intraperitoneal injection of ABT (50 mg/kg ip) to block the renal metabolism of AA by CYP450 enzymes, as previously described (27). Thirty-six hours later, the effects of an intrarenal infusion of S-NO-NAC were studied as described in protocol 4. At the end of each experiment, the kidneys were removed, and the degree of inhibition of the renal metabolism of AA by CYP450 enzymes produced by ABT was determined in microsomes prepared from the renal cortex using an HPLC-based radiochemical assay, as previously described (1, 28).
2. Briefly, microsomes (500 μg protein) were incubated at 37°C for 30 min in 1 ml of potassium phosphate buffer (0.1 M) containing sodium isocitrate (1 mM), isocitrate dehydrogenase (0.0016 U), [14C]AA (1 μCi), and NADPH (1 mM). The pH of the reaction was acidified to 3.5 with 1 M formic acid, samples were extracted twice with ethyl acetate, and the products were separated by reverse phase-HPLC. Products were monitored using a radioactive flow detector, and formation of 20-HETE, EETs, and DiHETEs was measured and expressed as picomoles per minute per milligram protein.

Table 1. Baseline measurements of MAP and renal function

<table>
<thead>
<tr>
<th>Group</th>
<th>MAP, mmHg</th>
<th>RBF, ml.min⁻¹.g⁻¹</th>
<th>GFR, μl.min⁻¹.g⁻¹</th>
<th>uV, μl.min⁻¹.g⁻¹</th>
<th>UₙNᵥ, μEq.min⁻¹.g⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>I (time control)</td>
<td>117 ± 2</td>
<td>116 ± 2</td>
<td>7.4 ± 0.7</td>
<td>7.5 ± 0.6</td>
<td>1,062 ± 42</td>
</tr>
<tr>
<td>2 (ODQ alone)</td>
<td>119 ± 3</td>
<td>118 ± 3</td>
<td>6.8 ± 0.6</td>
<td>6.7 ± 0.6</td>
<td>1,127 ± 26</td>
</tr>
<tr>
<td>3 (MB alone)</td>
<td>108 ± 6</td>
<td>109 ± 6</td>
<td>7.2 ± 0.8</td>
<td>7.1 ± 0.8</td>
<td>1,119 ± 135</td>
</tr>
<tr>
<td>4 (NO alone)</td>
<td>116 ± 1</td>
<td>119 ± 1*</td>
<td>7.3 ± 0.4</td>
<td>5.6 ± 0.4*</td>
<td>1,085 ± 61</td>
</tr>
<tr>
<td>5 (ODQ + NO)</td>
<td>112 ± 1</td>
<td>115 ± 2*</td>
<td>6.3 ± 0.5</td>
<td>4.3 ± 0.5*</td>
<td>1,151 ± 55</td>
</tr>
<tr>
<td>6 (MB + NO)</td>
<td>122 ± 5</td>
<td>130 ± 5*</td>
<td>7.4 ± 0.5</td>
<td>4.2 ± 0.5*</td>
<td>1,229 ± 57</td>
</tr>
<tr>
<td>7 (ABT + NO)</td>
<td>112 ± 3</td>
<td>114 ± 3</td>
<td>7.0 ± 0.9</td>
<td>5.1 ± 0.7</td>
<td>756 ± 61</td>
</tr>
<tr>
<td>8 (HET0016 + NO)</td>
<td>113 ± 4</td>
<td>114 ± 3</td>
<td>7.0 ± 0.7</td>
<td>6.1 ± 0.5*</td>
<td>1,014 ± 41</td>
</tr>
</tbody>
</table>

Values are means ± SE. Nos. at left indicate group; nos. at top indicate column. All drugs [except 1-aminobenzotriazole (ABT) and N-hydroxy-N'-4-butyl-2-methyl phenylformamide (HET0016)] were administered intrarenally. Before we performed the dose-response studies, groups 1–3 were infused with saline; groups 4–6 were infused with nitro-l-arginine methyl ester (L-NAME; 37 ng.kg⁻¹.min⁻¹) together with saline, 1H-1,2,4oxadiazolo[4,3-a]quinoxaline-1-one (ODQ; 170 nmol.kg⁻¹.min⁻¹), or methylene blue (MB; 170 nmol.kg⁻¹.min⁻¹); and groups 7 and 8 were pretreated with ABT (50 mg/kg ip) and HET0016 (10 mg/kg iv) before the experiment and infused with L-NAME (37 ng.kg⁻¹.min⁻¹). MAP, mean arterial pressure; RBF, renal blood flow; GFR, glomerular filtration rate; UV, urine flow; UₙNᵥ, sodium excretion; NO, nitric oxide.*Significant difference from the control period of the same group.
values in columns 4, 6, 8, and 10 of Table 1. MB had no significant effects on MAP. Intrarenal infusion of increasing doses of MB had effects similar to ODQ and produced graded reductions in RBF, GFR, and sodium and water excretion. The largest effects were seen after administration of the 1,700-nmol kg⁻¹ min⁻¹ dose (decreases of 57, 79, 55, and 53%, respectively).

Groups 4, 5, and 6: effects of ODQ or MB on renal response to L-NAME and an NO donor. A comparison of the effects of intrarenal infusion of the NO donor in L-NAME-treated rats receiving vehicle, ODQ, or MB is presented in Fig. 3. The absolute values for the control periods in Fig. 3 correspond to the values in columns 4, 6, 8, and 10 of Table 1. Intrarenal infusion of the NO donor S-NO-NAC at 5 and 50 nmol kg⁻¹ min⁻¹ produced significant increases in RBF (+14 and +18%), U₆₅N (±49 and +73%), and UV (+52 and +61%) in rats pretreated with L-NAME and vehicle (group 4). S-NO-NAC had no significant effect on arterial pressure or GFR when infused intrarenally at these doses. Intrarenal infusion of ODQ or MB blocked the effects of the NO donor on RBF, but the diuretic and natriuretic responses to S-NO-NAC were significantly enhanced.

Groups 4, 7, and 8: effect of ABT or HET0016 on renal response to l-NAME and an NO donor. A comparison of the effects of intrarenal infusion of the NO donor in l-NAME-treated rats receiving vehicle (group 4), ABT (group 7), or HET0016 (group 8) is presented in Fig. 4. The absolute values for the control periods in Fig. 4 correspond to the values in columns 4, 6, 8, and 10 of Table 1. Inhibition of CYP450 metabolism of AA with ABT or HET0016 also blocked the renal vasodilatory response to the NO donor and markedly attenuated the diuretic and natriuretic responses to intrarenal infusion of S-NO-NAC. The effects of ABT on the renal production of CYP450 products of AA are presented in Fig. 2B. The generation of DiHETEs, 20-HETE, and EETs by renal cortical microsomes fell by 75, 84, and 85%, respectively, 36 h after pretreatment of the rats with ABT. Similarly, a high concentration of HET0016 (1 μM), 100 times greater than the reported IC₅₀ for this compound (16), selectively reduced the formation of 20-HETE by renal cortical microsomes from 96 ± 3.9 to 0.86 ± 0.7 pmol min⁻¹ mg⁻¹ protein (n = 4), but it had no effect on the formation of EETs or DiHETEs (Fig. 2C).

DISCUSSION

The present study evaluated the relative contributions of cGMP and CYP450 metabolites of AA to the renal effects of an intrarenal infusion of an NO donor in rats. Infusion of the NO donor S-NO-NAC into the renal artery of rats pretreated with l-NAME to block the endogenous formation of NO increased RBF and sodium and water excretion, but it had no effect on MAP or GFR. These findings are consistent with previous results reported in dogs (13) and rats (22) infused intrarenally with NO donors or endothelium-dependent vasodilators (10, 23, 24). Pretreatment of rats with ODQ or MB blocked the effects of the NO donor on RBF. In contrast, ODQ and MB enhanced, rather than
inhibited, the diuretic and natriuretic response to S-NO-NAC. The potentiation of the diuretic and natriuretic effects of NO after blockade of GC was not due to differences in baseline UNaV. As can be seen in Table 1, baseline sodium and water excretion after treatment with l-NAME was similar in rats given vehicle (group 4), ODQ (group 5), or MB (group 6). These results indicate that the renal vasodilatory response to NO is cGMP dependent or that cGMP has an obligatory permissive role in this response. On the other hand, the diuretic and natriuretic actions of NO are likely mediated by a cGMP-independent action of NO. Overall, these results are consistent with previous findings indicating that cGMP contributes to the vascular relaxation induced by endothelium-dependent vasodilators, such as acetylcholine or bradykinin, that are known to increase the endogenous synthesis of NO (11, 23). Indeed, Lahera et al. (9) suggested that the actions of l-NAME on arterial pressure and renal hemodynamics are mediated by decreased availability of cGMP, because exogenous administration of 8-BrcGMP reverses these effects in rats in vivo. However, our finding that GC inhibitors completely block the vasodilatory response to intrarenal infusion of NO donors in vivo conflicts with the other reports indicating that NO elicits both cGMP-dependent and -independent effects on vascular tone in nonfiltering kidneys (29), and that the vasodilator response to NO in isolated renal vessels is largely cGMP independent (2) and mediated by inhibition of 20-HETE formation.

We therefore examined the contribution of CYP450 metabolites of AA to the renal actions of an NO donor. S-NO-NAC was given to rats pretreated with ABT or HET0016 to block the renal metabolism of AA by CYP450 enzymes. In this regard, Su et al. (27) recently reported that ABT (50 mg/kg ip) was a potent inhibitor of the metabolism of AA by CYP450 enzymes that selectively inhibits the formation of 20-HETE and diminishes the expression of CYP450A4 protein in the kidney (27). In the present study, a single dose of ABT (50 mg/kg ip) given 36 h before the acute experiment reduced the renal formation of 20-HETE and EETs by >80%. Pretreatment of rats with ABT completely blocked the effects of an intrarenal infusion of the NO donor to increase RBF. ABT also attenuated the diuretic and natriuretic response to the intrarenal infusion of the NO donor. These results are consistent with the view that the renal vasodilator responses to NO are secondary to inhibition of the formation of CYP450 metabolites of AA. They are also in complete agreement with the results of previous in vitro studies performed on renal interlobular arteries (1, 2, 18, 28).

The results obtained with ABT, however, do not allow one to determine which CYP450 metabolite of AA contributes to the renal response to NO, since ABT blocked the formation of both 20-HETE and EETs by 10.2 ± 0.3 mmol/L [S-NO-NAC]. concentration of S-NO-NAC. *Significant difference from control. B: effect of pretreatment of rats with 1-aminobenzotriazole (ABT; 50 mg/kg) on the metabolism of arachidonic acid (AA) by rat renal cortical microsomes. *Significant difference from control. C: effects of addition of N-hydroxy-N'-4 butyl-2-methyl phenyl)formamidine (HET0016; 1 μM) on the metabolism of AA by microsomes prepared from the renal cortex of a Sprague-Dawley rat. *Significant difference from control. B and C: 20-HETE, 20-hydroxyeicosatetraenoic acid. Epoxygenase activity refers to the sum of the production of epoxyeicosatrienoic (EETs) and dihydroxyeicosatetraenoic acids (DiHETEs).
with ABT. HET0016 blocked the renal vasodilator response to intrarenal infusion of an NO donor, attenuated the diuretic and natriuretic response to NO, and completely blocked the antinatriuretic response to L-NAME. Together, these results suggest that the renal hemodynamic and natriuretic responses to NO are probably related to inhibition of the renal formation of 20-HETE and not EETs.

Fig. 3. Effect of S-NO-NAC on RBF, GFR, UV, and U\textsubscript{Na}V in rats treated with ODQ (170 nmol·kg\textsuperscript{-1}·min\textsuperscript{-1}), MB (170 nmol·kg\textsuperscript{-1}·min\textsuperscript{-1}), or vehicle. The absolute values for the control periods correspond to the values in columns 4, 6, 8, and 10 of Table 1. ●, S-NO-NAC (n = 11); ○, ODQ + S-NO-NAC (n = 9); ▼, MB + S-NO-NAC (n = 6). *Significant difference from the control period within the same group. †Significant difference from the corresponding value in the vehicle-treated group.

Fig. 4. Effect of S-NO-NAC on RBF, GFR, UV, and U\textsubscript{Na}V in rats treated with ABT (50 mg/kg), HET0016 (10 mg/kg), or vehicle. The absolute values for the control periods correspond to the values in columns 4, 6, 8, and 10 of Table 1. ●, S-NO-NAC (n = 11); ▲, ABT + S-NO-NAC (n = 8); ○, HET0016 + S-NO-NAC (n = 6). *Significant difference from the control period within the same group. †Significant difference from the corresponding value in the vehicle-treated group.
Thus activation of $K^+$ channels in vascular smooth muscle (VSM) cells. According to this hypothesis, blockade of cGMP synthesis with ODQ or MB should enhance basal renal vascular tone by depolarizing VSM cells by blocking $K^+$ channels. Under these conditions, NO-induced inhibition of 20-HETE formation may be unable to enhance $K^+$ channel activity and reduce vascular tone. Blockade of cGMP formation is also known to enhance the contractile response to intracellular $Ca^{2+}$. Thus activation of $K^+$ channels and decreases in intracellular $Ca^{2+}$ concentration after NO inhibition of 20-HETE formation might not be able to reduce vascular tone when cGMP levels are low (3). Similarly, blockade of 20-HETE formation with ABT or HET0016 would be expected to increase $K^+$ channel activity, as is the case with 17-octadecynoic acid (17-ODYA) or N-methylsulfonyl-12,12-dibromododec-11-enamide (DDMS) (28), and to lower intracellular $Ca^{2+}$ levels by reducing $Ca^{2+}$ influx through L-type $Ca^{2+}$ channels (12). Under these conditions, NO-induced elevations in cGMP levels may have little effect to open $K^+$ channels, reduce intracellular $Ca^{2+}$ levels, or alter vascular tone. Thus the present findings are consistent with the view that both intact cGMP and 20-HETE systems are probably required to elicit a vasodilator response to NO.

In the present study, inhibition of GC activity with ODQ or MB did not affect the natriuretic and diuretic response to intrarenal infusion of the NO donor. This finding was unexpected, since cGMP is thought to mediate both the hemodynamic and the tubular actions of NO. One possible explanation is that ODQ may not be filtered in the kidney, and, therefore, tubular cGMP formation was not inhibited. However, this possibility seems remote, since we found that the ability of NO to stimulate cGMP production in renal cortical homogenates (largely proximal tubules) was blocked in rats infused with ODQ. Also, MB is rapidly excreted in urine (which rapidly becomes blue during the intrarenal infusion), indicating that it is filtered. Thus the results of the present study suggest that inhibition of tubular sodium reabsorption after intrarenal infusion of an NO donor is probably mediated by a cGMP-independent effect.

The question then arises as to whether the natriuretic response to NO is dependent on the formation of CYP450 metabolites of AA. 20-HETE and EETs inhibit sodium transport in the proximal tubule and thick ascending limb of the loop of Henle (21). Thus NO-induced inhibition of 20-HETE production would be expected to promote sodium retention rather than increase $U_{Na}V$. Also, the effect of L-NAME to reduce UV and $U_{Na}V$, as it has previously been reported (5) and was confirmed in the present study (Table 1), is incompatible with the view that this response is mediated by enhanced 20-HETE production, since 20-HETE inhibits sodium reabsorption. However, L-NAME also reduces RBF and medullary blood flow, which lowers renal interstitial pressure and increases proximal tubular reabsorption (5). Thus the renal hemodynamic effects of L-NAME that enhance sodium reabsorption might predominate over any natriuretic effects associated with elevations in tubular 20-HETE levels. This possibility is also consistent with the present finding that HET0016 had a greater ability than ABT to block the renal vasodilator effects of the NO donor. If HET0016 was also able to prevent NO-induced changes in medullary blood flow, this could explain how it attenuated the natriuretic and diuretic response to the NO donor.

In summary, the results of the present study indicate that the renal vasodilator response to exogenous administration of an NO donor is dependent on both the activation of GC and the inhibition of CYP450. However, the natriuretic and diuretic responses to NO appear to be cGMP independent and are mediated in part by inhibition of the formation of CYP450 metabolites of AA.

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