Role of nitric oxide and cyclooxygenase-2 in regulating the renal hemodynamic response to norepinephrine

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López, Ruth, María T. Llinás, Francisco Roig, and F. Javier Salazar. Role of nitric oxide and cyclooxygenase-2 in regulating the renal hemodynamic response to norepinephrine. Am J Physiol Regul Integr Comp Physiol 284: R488–R493, 2003. First published September 19, 2002; 10.1152/ajpregu.00449.2002.—We have reported that the renal hemodynamic effects of norepinephrine (NE) are modulated by cyclooxygenase-2 (COX-2)-derived metabolites. Our main objective was to examine whether there is an interaction between nitric oxide (NO) and COX-2 in modulating the renal hemodynamic effects of NE. NE was infused at three doses to anesthetized dogs pretreated with vehicle (n = 8), a selective COX-2 inhibitor (nimesulide) (n = 6), an NO synthesis inhibitor [Nω-nitro-L-arginine methyl ester; L-NAME] (n = 8), or with nimesulide and L-NAME (n = 5). During NE infusion, PGE_2 excretion increased (125%) in the control group and did not change in the L-NAME-treated dogs. The simultaneous inhibition of NO and COX-2 potentiated to a greater extent the NE-induced renal vasoconstriction than inhibition of either NO or COX-2. The NE-induced renal vasoconstriction during NO and COX-2 inhibition was reduced (P < 0.05) by infusing an AT_1 receptor antagonist (n = 6). These results suggest that there is an interaction between NO and COX-2 in protecting the renal vasculature from the NE effects and that angiotensin II partly mediates the NE-induced renal vasoconstriction when NO synthesis and COX-2 activity are reduced.

METHODS

Experiments were performed in mongrel dogs of either gender (18–30 kg) with free access to tap water and fed with a normal sodium diet. Protocols were designed according to the “Guiding Principles for Research Involving Animals and Human Beings” (1a). Surgical preparation was performed in anesthetized dogs (30 mg/kg pentobarbital sodium) as described (14–16, 21, 23, 24). Catheters were placed in the femoral artery for measuring mean arterial pressure (MAP) and in the femoral vein for infusion of inulin, nimesulide, and additional anesthetic. The right renal artery was fitted with noncannulating electromagnetic flow probes, and the probes were connected to a flowmeter. Distal to the flow probe, a curved 23-gauge needle attached to polyethylene tubing was inserted into the renal artery and connected to a peristaltic pump for infusion of saline, NE, Nω-nitro-L-arginine methyl ester (L-NAME), or/and candesartan. A 45-min stabilization activity is mediated by NO. However, it remains to be examined whether this hypothesis is correct. This study evaluates whether NE enhances the production of COX-2-derived metabolites through an NO-dependent mechanism and whether the simultaneous inhibition of NO synthesis and COX-2 potentiates the renal hemodynamic effects of NE to a greater extent than the inhibition of either NO synthesis or COX-2 activity. The hypotheses were that 1) NO is involved in the NE-induced increase in COX-2 activity and 2) the simultaneous inhibition of NO synthesis and COX-2 will potentiate more the NE-induced renal vasoconstriction than the inhibition of either NO synthesis or COX-2. If the second hypothesis is correct, the greater renal vasoconstriction induced by NE could be second-ary not only to the reduction in NO and PG but also to the hemodynamic effects elicited by the endogenous ANG II levels. This hypothesis is supported by studies showing that the renal hemodynamic effects of NE are partly mediated by ANG II (5, 20) and that the renal vasoconstriction induced by ANG II is potentiated when the production of NO and/or PG is reduced (1, 4, 15, 21, 27). Another objective of this study was to evaluate the role of endogenous ANG II levels in mediating the renal vasoconstriction induced by NE when NO synthesis and COX-2 activity are reduced.

renal adrenergic system; cyclooxygenases; AT_1 receptors antagonist; kidney
period was allowed before experimental maneuvers were begun.

Experimental Groups

Group 1 (n = 8). After two 15-min control clearances NE was infused into the renal artery at the rates of 50, 100, and 250 ng·kg⁻¹·min⁻¹. Each dose of NE was infused during 35 min, and one clearance was obtained during the last 20 min of each infusion.

Group 2 (n = 6). The experimental protocol was similar to that described for group 1 with the difference that nimesulide was infused as a bolus (0.75 mg/kg) and then continuously (5 μg·kg⁻¹·min⁻¹) for the duration of the experiment. The COX-2 selectivity of the dose of nimesulide used was demonstrated in a previous study (23).

Group 3 (n = 8). Forty-five minutes after initiating a continuous infusion of L-NAME (1 μg·kg⁻¹·min⁻¹), two 15-min control clearances were taken and the infusion of NE started afterward as in group 1. The infusions of the second and third doses were prolonged during 50 min, and the clearances were obtained during the last 35 min, because urine flow rate (UV) decreased very significantly.

Previous studies have reported findings suggesting that the dose of L-NAME used is effective in reducing NO synthesis (14–16, 24).

Group 4 (n = 5). L-NAME and nimesulide were simultaneously infused throughout the experiment at the doses used in groups 2 and 3. The two 15-min control clearances were initiated 45 min after these infusions started. Only the first two doses of NE were consecutively infused during 50 min with clearances obtained during the last 35 min of each infusion. The third dose was not infused because no urine flow was collected and renal blood flow (RBF) decreased to immeasurable levels.

Group 5 (n = 6). The experimental protocol was similar to that described for group 4, but candesartan was also infused into the renal artery as a bolus (0.5 mg/kg) and then continuously throughout the experiment (5 μg·kg⁻¹·min⁻¹). In preliminary experiments (n = 3) it was found that the fall in RBF (>70%) induced by an intrarenal ANG II infusion (12.5 ng/kg) was completely prevented during >120 min in dogs pretreated with this dose of candesartan.

Analytic Methods

Renal clearances were taken during each experimental period to determine the glomerular filtration rate (GFR), sodium excretion (UNaV), and UV. Blood samples for hematocrit, plasma sodium, and inulin concentrations were also obtained. GFR was measured by the clearance of inulin. Inulin concentrations were analyzed by the anthrone method. Sodium concentration was measured by flame photometry. Urinary excretion rate of PGE₂ was evaluated in groups 1 and 3. Urinary concentration of PGE₂ was measured with a commercial enzyme immunoassay (Cayman Chemical).

Statistical Analysis

The data for the two control clearance periods were averaged for statistical comparisons because the fluid and solute excretions were in steady-state conditions. Data are expressed as means ± SE. For each group, significant differences between values of each period were evaluated using ANOVA for repeated measures and the Fisher’s test. Significant differences among groups were calculated with the use of ANOVA and the Fisher’s test. A value of P < 0.05 was considered significant.

RESULTS

Group 1

MAP increased progressively (P < 0.05) from a control value of 135 ± 5 to 140 ± 5, 143 ± 6, and 148 ± 5 mmHg during the intrarenal infusion of the three NE doses. The renal hemodynamic response to NE is shown in Fig. 1. GFR only decreased (P < 0.05) during the infusion of the largest dose of NE (41 ± 3 vs. 26 ± 4 ml/min). It can be observed in Fig. 1 and Table 1 that NE elicited a dose-dependent fall in RBF (191 ± 12, 177 ± 12, and 136 ± 16 vs. 215 ± 11 ml/min in the basal period) and a dose-dependent rise in RVR. Filtration fraction (FF) only increased significantly during the intrarenal infusion of the second dose of NE (0.42 ± 0.01 vs. 0.35 ± 0.01% in the basal period, P < 0.05). Table 2 shows that UNaV decreased (P < 0.05) with the second and third doses of NE and that UV only decreased during the infusion of the greatest NE dose. Figure 2A shows that NE infusion caused a 125% increase (P < 0.05) in the urinary excretion rate of PGE₂.

Group 2

The NE infusion to nimesulide-pretreated dogs induced an elevation (P < 0.05) in MAP (from 133 ± 4 to 140 ± 5, 141 ± 5, and 146 ± 4 mmHg) that was similar to that found in vehicle-pretreated dogs. Figure 1 shows that NE induced a greater renal vasoconstriction in nimesulide than in the vehicle-pretreated dogs because GFR decreased (P < 0.05) with the first (35 ± 3 ml/min) and second (30 ± 3 ml/min) NE doses in dogs.
pretreated with nimesulide. The fall in GFR elicited by the greatest NE dose in this group (14 ± 3 vs. 42 ± 4 ml/min during the control period) was larger than that found in the control group (P < 0.05; Fig. 1). The fall in RBF (Fig. 1) and the rise in RVR (Table 1) induced by NE in dogs with a reduced COX-2 activity were similar to those found in the control group. FF did not change throughout the experiment, and both UNaV and UV only decreased (P < 0.05) with the largest dose of NE (Table 2).

**Group 3**

In dogs pretreated with l-NAME, MAP increased (P < 0.05) from 122 ± 4 to 133 ± 4 and 143 ± 3 mmHg during the infusion of the first two doses of NE. The increments in MAP elicited by NE in this group were not significantly different to those found in groups 1 and 2. The infusion of the greatest NE dose could not be finished in seven dogs because no urine flow was collected and RBF was not detectable in these dogs. GFR and RBF decreased with the intrarenal NE infusion to lower levels (P < 0.05) than those found in both the control and nimesulide-pretreated group (Fig. 1). GFR decreased (P < 0.05) from a basal value of 35 ± 4 to 24 ± 3 and 8 ± 4 ml/min with the first and second doses of NE. RBF decreased (P < 0.05) from a basal value of 197 ± 9 to 111 ± 11 and 57 ± 11 ml/min with these NE doses. As shown in Table 1, NE infusion in this group induced a greater rise in RVR (P < 0.05) than that induced by NE in groups 1 and 2. NE infusion in this group reduced (P < 0.05) UV and UNaV (Table 2). FF did not change significantly throughout the experiment. Figure 2 shows that the increment in the urinary excretion rate of PGE2 elicited by NE in the vehicle-treated dogs was abolished in the l-NAME-pretreated dogs.

**Group 4**

MAP increased from 139 ± 4 to 158 ± 7 and 164 ± 8 mmHg (P < 0.05) with the first and second doses of NE in dogs pretreated with l-NAME and nimesulide. The NE-induced increments in MAP were greater (P < 0.05) than those found in groups 1, 2, and 3. During NE infusion, GFR decreased from 30 ± 4 ml/min to levels (3 ± 1 and 2 ± 1 ml/min, respectively) that were lower (P < 0.05) than those found in dogs pretreated with vehicle, l-NAME, or nimesulide (Figs. 1 and 3). RBF decreased (P < 0.05) from 141 ± 16 to 39 ± 8 and 15 ± 5 ml/min during the infusion of the first two doses of NE (Fig. 3). As shown in Table 1, the rise in RVR

### Table 1. Changes in renal vascular resistance in response to intrarenal infusion of three doses of NE in dogs pretreated with vehicle, nimesulide, l-NAME, nimesulide and l-NAME, and in dogs pretreated simultaneously with nimesulide, l-NAME and candesartan

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>50</th>
<th>100</th>
<th>250</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>0.64 ± 0.03</td>
<td>0.76 ± 0.05*</td>
<td>0.84 ± 0.06*</td>
<td>1.23 ± 0.16*</td>
</tr>
<tr>
<td>Nimesulide</td>
<td>0.72 ± 0.02</td>
<td>0.81 ± 0.04*</td>
<td>0.87 ± 0.04*</td>
<td>1.64 ± 0.32*</td>
</tr>
<tr>
<td>l-NAME</td>
<td>0.63 ± 0.04</td>
<td>1.30 ± 0.14*</td>
<td>3.25 ± 0.54*</td>
<td>–</td>
</tr>
<tr>
<td>l-NAME + nimesulide</td>
<td>1.05 ± 0.12</td>
<td>7.62 ± 3.71*</td>
<td>17.8 ± 4.34*</td>
<td>–</td>
</tr>
<tr>
<td>l-NAME + nimesulide + candesartan</td>
<td>0.85 ± 0.07</td>
<td>0.94 ± 0.08*</td>
<td>1.99 ± 0.51*</td>
<td>6.83 ± 2.41*</td>
</tr>
</tbody>
</table>

Values are means ± SE. UNaV, urinary sodium excretion; UV, urine flow rate. *P < 0.05 vs. basal period.

### Table 2. Changes in UNaV and UV during intrarenal infusion of three doses of NE in dogs pretreated with vehicle, nimesulide, l-NAME, nimesulide and l-NAME, and in dogs pretreated simultaneously with nimesulide, l-NAME and candesartan

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>50</th>
<th>100</th>
<th>250</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>UNaV, μeq/min</td>
<td>57 ± 13</td>
<td>40 ± 9</td>
<td>31 ± 8*</td>
</tr>
<tr>
<td></td>
<td>UV, ml/min</td>
<td>0.43 ± 0.12</td>
<td>0.40 ± 0.10</td>
<td>0.41 ± 0.12</td>
</tr>
<tr>
<td>Nimesulide</td>
<td>UNaV, μeq/min</td>
<td>33 ± 9</td>
<td>23 ± 8</td>
<td>21 ± 9</td>
</tr>
<tr>
<td></td>
<td>UV, ml/min</td>
<td>0.17 ± 0.02</td>
<td>0.13 ± 0.02</td>
<td>0.12 ± 0.02</td>
</tr>
<tr>
<td>l-NAME</td>
<td>UNaV, μeq/min</td>
<td>22 ± 4</td>
<td>6 ± 2*</td>
<td>5 ± 2*</td>
</tr>
<tr>
<td></td>
<td>UV, ml/min</td>
<td>0.13 ± 0.03</td>
<td>0.09 ± 0.01</td>
<td>0.06 ± 0.01*</td>
</tr>
<tr>
<td>l-NAME + nimesulide</td>
<td>UNaV, μEq/min</td>
<td>14 ± 6</td>
<td>0.8 ± 0.4*</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>UV, ml/min</td>
<td>0.10 ± 0.03</td>
<td>0.02 ± 0.01*</td>
<td>–</td>
</tr>
<tr>
<td>l-NAME + nimesulide + candesartan</td>
<td>UNaV, μeq/min</td>
<td>12 ± 4</td>
<td>26 ± 7</td>
<td>21 ± 8</td>
</tr>
<tr>
<td></td>
<td>UV, ml/min</td>
<td>0.08 ± 0.02</td>
<td>0.14 ± 0.04</td>
<td>0.14 ± 0.05</td>
</tr>
</tbody>
</table>

Values are means ± SE. UNaV, urinary sodium excretion; UV, urine flow rate. *P < 0.05 vs. basal period.
elicited by NE was also greater than that found in groups 1, 2, and 3. UV and UNaV decreased in this group during NE infusion (Table 2). FF decreased from 0.41 ± 0.03 to 0.19 ± 0.08% during the administration of the first dose of NE.

**Group 5**

Contrary to what it was found in groups 1–4, MAP did not change during NE infusion in dogs pretreated with an AT1 receptor antagonist (from 131 ± 5 to 130 ± 5, 133 ± 4, and 132 ± 6 mmHg during the infusion of the 3 doses of NE). Renal clearances were not measured in four dogs during the administration of the greatest dose of NE because no urine flow was collected in these dogs. It can be observed in Fig. 3 that the renal vasoconstriction induced by NE was only partly but significantly prevented by the previous administration of an AT1 receptor antagonist. GFR decreased (P < 0.05) from 38 ± 3 to 34 ± 3 and 18 ± 5 ml/min during the intrarenal infusion of the first two doses of NE (Fig. 3). The fall in RBF (Fig. 3) and the increment in RVR (Table 1) elicited by NE in dogs pretreated with L-NAME and nimesulide were significantly reduced when the vasoconstrictor effects of ANG II were prevented with an AT1 receptor antagonist. UV and UNaV (Table 2) and FF did not change during the NE infusion in this group.

**DISCUSSION**

This is the first study suggesting that there is an interaction between NO and COX-2 in protecting the renal vasculature from the vasoconstriction elicited by NE and suggesting that the NE-induced activation of COX-2 is NO dependent. It is also proposed for the first time that the enhanced renal vasoconstriction induced by NE, when NO synthesis and COX-2 activity are reduced, is partly secondary to the unrestrained vasoconstriction produced by endogenous ANG II levels.

As in several previous studies (14, 16, 23, 24), nimesulide was infused in this study to inhibit selectively COX-2. Because the dose of nimesulide used reduces significantly the urinary excretion rate of PGE2 and 6-keto-PGF1α, but not that of thromboxane B2 and 11-dehydro-thromboxane B2 (23), it is proposed that the nimesulide effects are secondary mainly to the inhibition of COX-2 activity. However, it cannot definitively rule out that COX-1 activity is not modified by nimesulide. The results obtained in this study confirm those reported by our group demonstrating that the renal vasoconstriction elicited by NE is proportional to the rise in NE levels and that COX-2-derived metabolites are involved in modulating the renal hemodynamic effects of NE (14). The involvement of COX-2 was demonstrated in studies showing that nonselective COX inhibition or selective COX-2 inhibition potentiates to the same extent the renal vasoconstriction elicited by NE (14). It was also found that selective COX-2 inhibition prevents the increment in the urinary excretion rate of PGE2 and 6-keto-PGF1α elicited by NE (14).

It has also been suggested that NE increases NO synthase expression and activity (9, 18) and that NO plays an important role in modulating the vasoconstrictor actions of NE (7, 8). Considering that it has been proposed that NO enhances renal COX-2 expression and activity under different experimental situations (3, 11, 24, 26), the present studies examine whether NO is involved in increasing the production of the COX-2-derived PG that modulate the renal vasoconstriction elicited by NE. The hypothesis that the NE-induced activation of COX-2 is mediated by NO appears to be correct because it was found in this study that the NE-induced increment in urinary PGE2 excretion is prevented in dogs pretreated with L-NAME. In a previous study (14) it was reported that this increment...
in PGE2 is also prevented when the dogs are pretreated with nimesulide. The protective effect of NO on the renal vasoconstriction induced by NE seems to be more important than that elicited by COX-2-derived metabolites as we found that the pretreatment with L-NAME potentiates to a greater extent the NE-induced renal vasoconstriction than the pretreatment with nimesulide. However, the enhanced renal vasoconstriction observed in the L-NAME-pretreated dogs may be secondary not only to the reduction in NO but also to the prevention in the increment of COX-2-derived metabolites.

The efficacy of endogenous NO and COX-2-derived metabolites in protecting the renal vasculature from the effects elicited by NE depends on the increment in NE, being more important during infusion of the lowest than during infusion of the greatest dose of NE. The administration of the lowest NE dose only induced a small decrease (11%) of RBF in vehicle-treated dogs and a very important fall in GFR (91%) and RBF (72%) in dogs pretreated with L-NAME and nimesulide. However, the greatest NE dose already induced a decrease in GFR (37%) and RBF (37%) in vehicle-treated dogs. With these results we propose that a small increment in renal adrenergic activity does not modify significantly renal hemodynamics (5) because the endogenous levels of NO and PG modulate the NE effects. It is expected that the same small increment in renal adrenergic activity will lead to a very important renal vasoconstriction when both NO production and COX-2 activity are reduced.

The results of our study also suggest that there is an important interaction between NO and COX-2-derived PG in protecting the renal vasculature from the effects elicited by NE, because the decrease in GFR and RBF found in response to the NE infusion was greater during the simultaneous administration of L-NAME and nimesulide than when these inhibitors were administered separately. This interaction is more evident during the infusion of the lowest NE dose because it reduced GFR by 17% in nimesulide-treated dogs, by 32% in L-NAME-treated dogs, and finally reduced GFR by 91% in dogs treated with L-NAME and nimesulide. Neither the pathway by which this interaction occurs nor the mechanism by which NO and COX-2 interact can be derived from our results. However, it may be proposed that the primary compensatory vasodilator effects buffering NE-induced renal vasoconstriction during COX-2 inhibition are NO dependent. Because PG excretion did not change during NO inhibition in response to NE, it may also be suggested that the basal levels of COX-2-derived PG are more effective in protecting the renal vasculature when NO production is reduced than when it is not reduced. The existence of an interaction between NO and COX-2-derived PG in regulating renal function has been reported in several studies (2, 16, 24).

The greater renal vasoconstriction induced by NE in dogs treated with L-NAME and nimesulide could be secondary not only to the hemodynamic effects of NE but also to a hypersensitivity to the vasoconstrictor effects of the endogenous ANG II levels. The role of ANG II levels in mediating the vasoconstriction elicited by NE during the simultaneous inhibition of COX-2 and NO synthesis is supported by studies showing that NE enhances renin release (5) and that the renal hemodynamic effects of ANG II are modulated by NO and PG (4, 15, 27). It has also been reported that ANG II inhibition in rats pretreated with a COX inhibitor partly attenuated the effects of renal nerve stimulation on renal hemodynamics (20). Our results showing that the increment in MAP elicited by NE is completely prevented with the administration of an AT1 receptor antagonist support those reported by Reinhart et al. (22) in one study in which it was found that ANG II plays a critical role in mediating the hypertension associated with an elevation in NE.

Although endogenous ANG II does not completely account for the progressive renal constrictor response to NE, it plays an important role in mediating the renal vasoconstriction elicited by NE, when NO synthesis and COX-2 activity are reduced. This idea is supported by the results showing that the first two doses of NE reduced GFR and RBF to a greater extent in dogs in which ANG II effects were not modified than in those in which these effects were prevented by the administration of an AT1 receptor antagonist (Fig. 3). The greater decrease in RBF and GFR seems to be the result of removing intrinsic NO and PG-mediated vasodilatation, allowing the vasoconstrictor effects of NE and ANG II to predominate. Taken together, the results of this study present new evidence suggesting that there is an interaction between NO and COX-2-derived PG in protecting the renal vasculature not only from the vasoconstrictor effects of NE but also from those elicited by ANG II. The mechanism underlying this response cannot be elucidated from our data, but it has been shown that NO and PG are very effective in antagonizing the constriction of preglomerular arterioles and mesangial cells induced by NE and ANG II (6, 19, 20).

Intrarenal NE infusion induced not only a renal vasoconstriction but also a significant fall in renal excretory function. However, and contrary to the effects on renal hemodynamics, NO and COX-2-derived PG do not seem to modulate the effects of NE on sodium and water reabsorption because the fall in UNaV and UV elicited by NE is not greater in dogs pretreated with nimesulide and/or L-NAME. Pretreatment with nimesulide or L-NAME reduced basal values of UNaV and UV. This effect of COX-2 inhibition was expected because previous studies (14, 16, 23) have shown that COX-2 inhibition reduces the renal excretory ability. However, the decrease in UNaV and UV during pretreatment with L-NAME was unexpected because in previous studies of our group (15, 16) it was found that the acute intrarenal L-NAME infusion at the dose used in this study has no effect on renal function. These results show that the administration of a low dose of L-NAME may reduce UNaV and UV without affecting renal hemodynamics and confirm those reported by Lahera et al. (13).
In summary, our results suggest that the kidney may become very susceptible to small elevations in renal adrenergic activity when NO production and COX-2 activity are reduced. Our findings could have clinical implications regarding situations associated with an increase in renal adrenergic activity and a process such as aging, which seems to be associated with endothelial dysfunction (17) and in which the use of anti-inflammatory drugs is quite frequent (10).

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REFERENCES


