Role of Nitric Oxide and Prostaglandins in the Long-term Control of Renal Function

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Abstract—Previous studies have reported evidence of an important interaction between nitric oxide (NO) and prostaglandins in the acute regulation of renal function. The objective of this study was to determine in conscious dogs whether the renal effects of the prolonged administration of a cyclooxygenase inhibitor are enhanced when NO synthesis is reduced. Meclofenamate infusion (5 μg \cdot kg^{-1} \cdot min^{-1}) during 4 consecutive days (n=8) elicited a continuous decrease (P<0.05) in renal blood flow and plasma renin activity and a transitory decrease in sodium excretion. N^{G}-Nitro-L-arginine methyl ester (L-NAME) infusion (5 μg \cdot kg^{-1} \cdot min^{-1}) during 6 days (n=8) produced a significant increase in arterial pressure and a transitory decrease (P<0.05) in both renal blood flow and plasma renin activity. The simultaneous inhibition of NO and prostaglandin synthesis (n=7) led to an increase in arterial pressure and a decrease in renal blood flow similar to those observed during the administration of either L-NAME or meclofenamate. In contrast, this simultaneous inhibition produced a decrease in glomerular filtration rate, which was not observed in the previous groups, and also induced an increase in renal vascular resistance and a decrease in sodium excretion greater (P<0.05) than those found during the inhibition of either NO or prostaglandins. Only a transitory decrease in plasma renin activity was found during meclofenamate infusion in this group. The results of this study present new evidence that the renal vasoconstrictor and antinatriuretic effects induced by the prolonged infusion of a cyclooxygenase inhibitor are significantly enhanced when NO synthesis is reduced. These results suggest that renal function may be more sensitive to the prolonged administration of a cyclooxygenase inhibitor in situations where NO production is reduced. (Hypertension. 1998;32:33-38.)

Key Words: vasoconstriction ■ nitric oxide ■ prostaglandins ■ sodium ■ plasma renin activity

The role of NO and PG in the acute and long-term regulation of arterial pressure and renal function has been examined in many studies (see References 1 through 4 for review). It has been reported that acute and long-term NO synthesis inhibition induces a significant elevation in arterial pressure and RVR and a decrease in the renal excretory ability.1–3 The results obtained during the acute administration of a cyclooxygenase inhibitor are contradictory, but it is generally accepted that it can induce small changes in RVR and sodium excretion.2,4 The effects of the prolonged PG synthesis inhibition on renal function have been evaluated, but changes in RBF during the first days of cyclooxygenase inhibition have been only partly examined.2,4

Results obtained in previous studies performed in anesthetized dogs suggest that an important interaction exists between NO and PG in the acute regulation of renal hemodynamic and excretory function.5,6 It was found that the renal effects induced by acute intrarenal L-NAME administration are significantly potentiated when PG synthesis is reduced. However, it is not known whether there is an interaction between NO and PG in the prolonged regulation of renal function. The objective of this study was to determine, in conscious chronically instrumented dogs, whether the effects induced by the prolonged administration of a cyclooxygenase inhibitor on arterial pressure and renal function are enhanced when NO synthesis is reduced. The results obtained in this study may have important clinical implications because, during aging and in sodium-sensitive hypertension, NO synthesis seems to be reduced7 and the intake of nonsteroidal antiinflammatory drugs is frequent.8

Methods

Animal Preparation

Experiments were performed in 29 female conscious chronically instrumented dogs (16 to 22 kg). Surgery was performed under aseptic conditions, and the experiments were designed according to the Guiding Principles in the Care and Use of Laboratory Animals approved by the Council of the American Physiological Society. The dogs were surgically instrumented under anesthesia induced with pentobarbital (0.4 mL/kg) and maintained with a 1.5% to 2% halothane/O_2 mixture. Tygon catheters were inserted through the femoral vessels into the abdominal aorta, distal to the origins of the renal arteries, and the inferior vena cava. The arterial catheter was used for arterial pressure monitoring and blood sample collections, and the venous catheter was used for infusion of various solutions. A transit-time flow probe (4R, Transonic Systems) was implanted on the left renal artery for the measurement of RBF. The catheters and cable connected to the probe were tunneled subcutaneously, exter-
orized between the scapulae, and placed in neck collars. The dogs were allowed to recover from surgery for at least 2 weeks before any experiments were performed. Antibiotic prophylaxis was initiated before surgery and maintained throughout the experiment.

Seven days before the experiments were started, the dogs were housed in individual metabolic cages and fitted with harnesses that contained arterial pressure transducers mounted at heart level and a connector to the flow line. The arterial pressure and flow lines were connected to an analog-to-digital data collection system (Transonic, No. T208) and the data obtained analyzed using an IBM personal computer. Use of the transit-time flowmeter has been demonstrated to be a good method to determine continuous changes in blood flow.6 During the experiments, the data from the recorder were obtained every minute and subsequently averaged over a 20-hour period (12 PM to 8 AM).

Dogs were fed a sodium-deficient diet (H/D, Hill Pet Products) that provided 5 to 7 mmol of sodium per day and were allowed free access to tap water throughout the experiment. The venous catheter was connected to a peristaltic pump for continuous infusion of isotonic saline to maintain sodium intake constant at approximately 80 mmol/d. Saline was pumped through a disposable filter (0.22 μm, Cathivex, Millipore) to prevent the formation of air bubbles and possible contaminants from entering the venous system. The filters were changed frequently throughout the study. The infusion tubing and both the pressure and flow lines were protected with a flexible vacuum hose that was attached to the harness. The dogs were allowed to move freely in the cage but were unable to turn around completely.

Experimental Groups

**Group 1 (n=6)**

Only isotonic saline was infused throughout the experiment and 24 urine samples were measured between 8:30 and 9 AM each day. Samples for the measurement of GFR (24-hour creatinine clearance), plasma sodium and potassium concentrations, osmolality, and hematocrit were drawn daily, 22 hours after the last feeding. In addition, blood samples were obtained on days 1, 4, 7, 8, and 10 of the experiment to analyze PRA.

**Group 2 (n=8)**

After a control period of 3 days, a continuous IV infusion of meclofenamate (5 μg·kg⁻¹·min⁻¹) was started and maintained during 4 consecutive days. A recovery period of 3 days was allowed after the meclofenamate infusion was finished. Urinary and blood samples were obtained on days 1, 4, 7, and 10 of the control period (days 1), on the first and last days of meclofenamate infusion (days 4 and 7), and during the recovery period (days 8 and 10).

In preliminary experiments (n=6), it was found that meclofenamate infusion during 6 consecutive days, at the dose used in this study, reduced the urinary excretion of PGE₂ and 6-keto-PGF₁α. These PGs were measured using a commercially available radioimmunoassay (New England Nuclear). The urinary excretion rate of PGE₂ and 6-keto-PGF₁α decreased (P<0.05) from 172±18 and 443±44 pg/min in the control period to 52±12 and 40±3 pg/min, respectively, on the third day of meclofenamate administration. No side effects (eg, diarrhea or vomiting) were observed in these experiments during the first 4 days of meclofenamate infusion. Only 2 dogs had diarrhea by the sixth day of meclofenamate infusion.

**Group 3 (n=8)**

L-NAME (5 μg·kg⁻¹·min⁻¹ IV) was infused during 6 consecutive days after a control period of 3 days. A recovery period of 3 days was allowed after the L-NAME infusion was finished. Urinary and blood samples were taken in the same fashion as those obtained in group 1, with the difference that blood samples to analyze PRA were drawn during the control period (day 1), on the first and last days of L-NAME infusion (days 4 and 9), and during the recovery period (days 10 and 12).

**Group 4 (n=7)**

A similar protocol to that of group 3 was performed, with the difference that a meclofenamate infusion (5 μg·kg⁻¹·min⁻¹ IV) was started 48 hours after that of L-NAME (5 μg·kg⁻¹·min⁻¹ IV). L-NAME and meclofenamate administration was maintained for 6 and 4 days, respectively. The objective was to reduce only NO synthesis during 2 days (days 4 and 5) and to reduce simultaneously NO and PG synthesis during 4 consecutive days (days 6 through 9). A recovery period of 3 days was allowed after the infusion of L-NAME and meclofenamate was finished. Plasma samples to analyze PRA were drawn during the control period (day 1), on the first day of L-NAME infusion (day 4), on the first and last days of the simultaneous inhibition of NO and PG synthesis (days 6 and 9), and during the recovery period (days 10 and 12).

### Analytic Methods

Sodium and potassium concentrations in the urine and plasma were measured by flame photometry (Instrumentation Laboratories). GFR was determined by the clearance of endogenous creatinine. Creatinine was measured with a photocolorimetric method (Boehringer-Mannheim). PRA was measured using a commercially available radioimmunoassay (RENCTK P2721, Sorin Biomedica). RVR was calculated as the ratio of MAP to RBF.

### Statistical Analysis

Data are expressed as mean±SE. Significance of differences in values of each day compared with the control period was evaluated using a one-way ANOVA for repeated measures and the Fisher test for multiple comparisons. The significant difference between the same experimental day in different groups was calculated with a two-way ANOVA and the Duncan test. P<0.05 was considered significant.

### Results

**Group 1**

It can be observed in Figures 1 and 2 that, as expected, MAP, GFR, RBF, and UNaV did not change when only isotonic saline was infused. No significant changes were found throughout the experiment in PRA (Figure 3), UV, hematocrit, and plasma sodium and potassium concentrations.

**Group 2**

Figure 1 illustrates the effects of meclofenamate infusion on MAP, GFR, and RBF. It can be observed that MAP and GFR did not change throughout the experiment and that meclofenamate infusion induced a continuous decrease in RBF (P<0.05) that remained significant even during the 3 days of recovery. RVR increased from 0.44±0.11 (control period) to 0.53±0.12 and 0.66±0.10 mm Hg · mL⁻¹ · min⁻¹ on the first and last days, respectively, of meclofenamate infusion. UNaV decreased (P<0.05) on only the first and last days of
meclofenamate administration and returned to control levels (81 ± 5 mmol/d) during the recovery period (Figure 2). The response of UV was similar to that of UNaV. PRA decreased (P < 0.05) on the first and last days of meclofenamate infusion and increased to control levels (0.7 ± 0.1 ng Ang I · mL⁻¹ · h⁻¹) thereafter (Figure 3). As expected, plasma sodium concentration did not change and plasma potassium levels increased (P < 0.05) with meclofenamate administration (5.0 ± 0.1 versus 4.4 ± 0.1 mmol/L in the control period). Plasma potassium remained slightly elevated (P < 0.05) on the third day of the recovery period (4.7 ± 0.1 mmol/L). Hematocrit did not change throughout the experiment.

**Group 3**

Figure 4 shows the changes in MAP, GFR, and RBF induced by L-NAME. MAP increased (P < 0.05) from 98 ± 4 to 110 ± 5 mm Hg on the first day and remained elevated during the 6 days that NO synthesis was reduced. GFR did not change throughout the experiment, and RBF decreased (P < 0.05) on only the first day of L-NAME infusion (225 ± 18 versus 251 ± 10 mL/min in the control period) (Figure 4). UNaV (Figure 2), UV, hematocrit, and plasma sodium and potassium concentrations did not change throughout the experiment. Figure 3 shows that PRA decreased (P < 0.05) on the first day of L-NAME infusion (0.2 ± 0.1 ng Ang I · mL⁻¹ · h⁻¹) and then increased to levels not different from those found in the control period (0.8 ± 0.2 ng Ang I · mL⁻¹ · h⁻¹).

**Group 4**

It can be observed in Figures 2 and 4 that the response of MAP, GFR, RBF, and UNaV to L-NAME infusion during 2 days is similar to that observed in group 3. The simultaneous NO and PG synthesis inhibition induced an increase in MAP that was similar to that elicited by the inhibition of NO alone (Figure 4) and a decrease in RBF that was similar to that produced by the reduction of PG synthesis alone (Figures 1 and 4). However, the increment of RVR in this group was greater (P < 0.05) during the 4 days that PG and NO synthesis were simultaneously reduced (0.73 ± 0.05 versus 0.40 ± 0.03 mm Hg · mL⁻¹ · min⁻¹ in the control period) than in the groups in which only NO or PG synthesis was inhibited. Another important difference regarding the results found during the inhibition of either NO or PG synthesis is that there was a significant decrease (P < 0.05) in GFR during both the third (31 ± 5 mL/min) and fourth (29 ± 7 mL/min) days of the simultaneous NO and PG synthesis reduction (Figure 4). GFR increased to control levels (45 ± 6 mL/min) during the first day of the recovery period but decreased again (P < 0.05) on the second and third days of this period (Figure 4).

Figure 2 shows that UNaV remained decreased during the 4 days that NO and PG synthesis were reduced and returned to control levels during the recovery period. Cumulative sodium balance during the 4 days of meclofenamate infusion was greater (P < 0.05) in this group (148 ± 24 mmol) than in group 2 (54 ± 18 mmol). The changes in UV were similar to those of UNaV. As shown in Figure 5, PRA decreased (P < 0.05) on the first day of the simultaneous NO and PG synthesis inhibition (0.2 ± 0.1 ng Ang I · mL⁻¹ · h⁻¹) and
returned to control levels (0.8±0.2 ng Ang1· mL−1· h−1) on the last day of L-NAME and meclofenamate infusion. Plasma sodium concentration did not change throughout the experiment. Plasma potassium levels increased (P<0.05) during simultaneous NO and PG synthesis reduction (4.9±0.1 versus 4.2±0.1 mmol/L) and remained elevated (P<0.05) during the recovery period (4.7±0.1 mmol/L). Hematocrit was diminished (0.31±0.01) during the last day of the simultaneous L-NAME and meclofenamate administration compared with the control period (0.35±0.01, P<0.05).

Discussion

This study reports for the first time that the renal vasoconstrictor and antinatriuretic effects induced by the prolonged administration of a cyclooxygenase inhibitor are enhanced when NO production is reduced. The simultaneous inhibition of NO and PG synthesis induced an increase in RVR and a decrease in GFR and sodium excretion that were greater than those induced by the reduction in either NO or PG synthesis alone. Another novel finding is that the administration of a cyclooxygenase inhibitor does not produce a prolonged decrease in PRA when NO synthesis is reduced. Finally, this is the first study in which RBF has been continuously measured during the first days of administering a cyclooxygenase inhibitor. It was observed that the prolonged PG synthesis inhibition induces a significant decrease in RBF.

The role of endogenous PG in the acute and long-term regulation of arterial pressure and renal function has been evaluated in many studies by the administration of cyclooxygenase inhibitors (see References 2 and 4 for review). It has been observed that acute PG synthesis inhibition does not induce changes in arterial pressure but may produce an increase in RVR and a decrease in sodium and water excretion.5,6,10 In this study, the prolonged PG synthesis inhibition induced changes in renal excretory function, PRA, and plasma potassium levels that were similar to those reported in studies in which a cyclooxygenase inhibitor was administered during several days in animals or humans with a normal sodium intake.2,4,11,12 However, the continuous de-

![Figure 3](image_url)

**Figure 3.** Changes in PRA during the administration of meclofenamate (MECLO, 5 μg·kg⁻¹·min⁻¹) or L-NAME (5 μg·kg⁻¹·min⁻¹) in both studies. *P<0.05 vs control period.

![Figure 4](image_url)

**Figure 4.** Changes in MAP, GFR, and RBF during the administration of L-NAME for 6 consecutive days (days 4 through 9) or the simultaneous administration of L-NAME (5 μg·kg⁻¹·min⁻¹, days 4 through 9) and meclofenamate (MECLO, days 6 through 9). *P<0.05 vs average of the 3-day control period.

![Figure 5](image_url)

**Figure 5.** Changes in PRA during the first and fourth days of simultaneous meclofenamate (MECLO, 5 μg·kg⁻¹·min⁻¹) and L-NAME (5 μg·kg⁻¹·min⁻¹) administration and the first and third days of the recovery period (RECOV). *P<0.05 vs control period.
crease in RBF found in this study during prolonged meclofenamate administration was unexpected because the dogs had a normal sodium intake (~80 mmol/d) and PRA was not elevated. These results suggest that endogenous PGs play an important role in the long-term regulation of RBF.

The effects of prolonged NO synthesis inhibition in conscious dogs with the administration of pressor doses of L-NAME have also been examined previously, with similar results to those obtained in this study. These results suggest that endogenous NO plays an important role in the regulation of arterial pressure and renal function and that the long-term pressure-natriuresis relationship is shifted to the right when NO synthesis is reduced. Although highly speculative because the glomerular hemodynamics have not been examined, the fact that RBF decreased transiently in our study without changes in GFR suggests that NO is more important in regulating the efferent than the afferent arteriole resistance. In support of this idea, it has been reported that immunoreactivity to NOS I and NOS III antibodies is greater in the efferent than in the afferent arteriole.

A weakness in this study is the lack of quantification of the amount of NO synthesis inhibition. This weakness is similar to that in many long-term studies performed with the administration of an NO synthesis inhibitor because there is not an easy way to evaluate the NO production in conscious animals. However, the dose used was high enough to elicit an increase in MAP and to enhance the renal vasoconstrictor and antinatriuretic effects induced by the infusion of a cyclooxygenase inhibitor. The hypertension and renal vasoconstriction induced by the prolonged L-NAME administration is most probably due to the reduction in NO synthesis because L-NAME infusion induces a decrease in NOS activity.

The present work is the first study showing the renal effects of prolonged systemic administration of a cyclooxygenase inhibitor in animals in which the systemic NO synthesis is diminished. The results obtained suggest that, even when NO synthesis is reduced, endogenous PGs do not play an important role in the maintenance of arterial pressure because the meclofenamate infusion did not induce a further increment in MAP in the L-NAME–treated dogs. However, the administration of this cyclooxygenase inhibitor produced a significant increase in RVR and a decrease in UNaV, UV, and GFR in these dogs. These results suggest that endogenous PGs modulate the antinatriuresis and renal vasoconstriction induced by the prolonged NO synthesis reduction. The greater renal vasoconstriction and increased sodium reabsorption elicited by the simultaneous administration of L-NAME and meclofenamate might be secondary to the decrease in NO and PG or to the effects of other agents, such as Ang II. The Ang II effects on renal vasculature and tubules would now be exerted without the modulating actions of NO and PG, thus leading to an increase in RVR and a decrease in sodium excretion. This concept is supported by the facts that PRA was normal during the L-NAME and meclofenamate administration and the renal hemodynamic and antinatriuretic Ang II effects are significantly potentiated when NO and PG synthesis are simultaneously decreased. Several studies have also demonstrated that the renal Ang II effects are enhanced when NO or PG synthesis is reduced. The important decrease in the renal ability to eliminate sodium and water during simultaneous L-NAME and meclofenamate administration suggests that the prolonged increment in sodium intake may induce the development of a sodium-sensitive hypertension in situations in which NO and PG synthesis are reduced. Indeed, it has been reported that NO synthesis inhibition induces the development of a sodium-sensitive hypertension.

The reported effects of prolonged NO synthesis inhibition on PRA levels are contradictory. The most frequent explanation given for the hyperkalemia induced by the cyclooxygenase inhibitors is a suppression of renin and aldosterone. However, the decrease in renin release and possible aldosterone suppression is not the only explanation for the hyperkalemia elicited by the administration of a cyclooxygenase inhibitor. In our study, it was found in dogs with a reduced NO synthesis that meclofenamate induces an increase in plasma potassium levels without changes in PRA. The mechanism responsible for this effect is not readily apparent, but it has been suggested that the hyperkalemia induced by the cyclooxygenase inhibitors could be secondary to a decrease in distal sodium delivery or to the activation of a high-conductance K+ channel that has been described in the collecting tubules.

To summarize, the results obtained in this study suggest that the renal hemodynamic and excretory functions may be more sensitive to the prolonged administration of a cyclooxygenase inhibitor in situations in which NO production is reduced. In support of this hypothesis, it has been observed that renal function declines promptly in elderly patients with hypertension and mild renal insufficiency; it is known that the intake of nonsteroidal antiinflammatory drugs is increased during aging and in hypertensive patients, and finally it has been suggested that NO production is reduced during aging and in salt-sensitive hypertension.

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References


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