Role of Cyclooxygenase-2 in the Prolonged Regulation of Renal Function

Francisco Roig, Maria T. Llinás, Ruth López, F. Javier Salazar

Abstract—The role of cyclooxygenase-2 (COX-2) in the prolonged regulation of renal function was evaluated during changes in sodium intake and reduction of NO synthesis. It was evaluated in conscious dogs by administering a selective inhibitor (nimesulide) during 8 consecutive days. Nimesulide administration to dogs with normal or high sodium load did not modify glomerular filtration rate but reduced renal blood flow (16%; \( P < 0.05 \)). The vasoconstriction elicited by COX-2 inhibition was greater when NO production was inhibited because glomerular filtration rate decreased by \( >25\% \) when nimesulide was administered to dogs with a reduced NO synthesis. During low sodium intake, COX-2 inhibition elicited a decrease \( (P < 0.05) \) of both glomerular filtration rate (34%) and renal blood flow (31%). Sodium excretion only decreased \( (P < 0.05) \) during the first day of COX-2 inhibition in dogs with normal or high sodium load. The increase in plasma potassium levels elicited by COX-2 inhibition was greater in dogs with low sodium intake and was enhanced when NO production was inhibited. This change in potassium was not secondary to a decrease in plasma aldosterone levels. The results of this study suggest that COX-2– derived metabolites (1) play a more important role in the long-term regulation of renal hemodynamic when sodium intake is low, (2) protect the renal vasculature from the vasoconstriction secondary to a reduction in NO, (3) are only acutely involved in regulating urinary sodium excretion, and (4) play a more important role in regulating plasma potassium concentration when NO synthesis is reduced. \( \text{Hypertension. 2002;40:721-728.} \)

Key Words: cyclooxygenase | nitric oxide | renal blood flow | hemodynamics | sodium | prostaglandins | renin | aldosterone

Numerous studies have demonstrated that cyclooxygenase-2 (COX-2) expression in the renal cortex increases in response to low-salt diet, and decreases when sodium intake is elevated.\(^1\)-\(^4\) These variations in COX-2 expression suggest that COX-2–derived metabolites play a major role in the control of renal hemodynamic when sodium intake is low, and probably a minor role in regulating renal hemodynamic when sodium intake is high. However, the role of COX-2 in the prolonged regulation of renal blood flow (RBF) and glomerular filtration rate (GFR) during changes in sodium intake is not well defined.\(^5\)-\(^8\) In the renal medulla, COX-2 expression increases in response to a high sodium intake.\(^3\),\(^4\) Considering the role of prostaglandins (PGs) in the control of sodium excretion,\(^9\) it may be proposed that COX-2 is more involved in regulating renal excretory function during high sodium intake than during normal sodium intake, but so far, it has not been evaluated whether this hypothesis is correct. The first objective of the present study was to evaluate the role of COX-2–derived metabolites in the prolonged regulation of renal hemodynamic and excretory function when sodium load is elevated or low.

The role of COX-derived PG in the regulation of plasma potassium (pK) and plasma aldosterone concentration (PAC) has been demonstrated in studies showing that pK and PAC are modified during the prolonged administration of a non-selective COX inhibitor.\(^10\)-\(^13\) However, it is unknown whether these PG are derived from COX-2. Our second objective was to examine the role of COX-2–derived metabolites in the long-term control of pK and PAC, and to assess whether this role changes when sodium intake is modified.

Previous studies\(^10\),\(^14\) have also proposed that endogenous PG modulate the renal vasoconstriction elicited by a reduction in NO synthesis. However, it has not been elucidated if these PG are COX-1– or COX-2–dependent. Our third objective was to examine the role of COX-2–derived PG in the prolonged regulation of renal function when NO synthesis is reduced. The studies to accomplish this objective were performed in dogs with normal and high sodium load, because it has been proposed that NO synthesis is enhanced during elevations in sodium load.\(^15\),\(^16\)

Methods

Experiments were performed in 35 female conscious instrumented dogs (16 to 24 kg). Surgery was performed under aseptic conditions, and the experiments were designed according to the Guiding Principles approved by the American Physiological Society. Dogs
were surgically instrumented as previously described\textsuperscript{10,11,15} for the continuous intravenous infusions and mean arterial pressure (MAP) and RBF measurements. MAP and RBF data were obtained every minute and subsequently averaged over a 20-hour period (1:00 PM to 9:00 AM). Dogs were fed a sodium-deficient diet (H/D, Hill Pet Products), which provided 4 to 6 mmol sodium/day, and were allowed free access to tap water throughout the experiment. The venous catheter was connected to a peristaltic pump for infusion of isotonic saline to maintain sodium load at \( \sim 70 \) or 200 mEq/d.

**Experimental Groups**

**Group 1 (n=7)**
Isotonic saline was infused at a rate of 425 mL/d to maintain a sodium load of 70 mEq/d. After a control period of 3 consecutive days, nimesulide was given orally during 8 consecutive days (5 mg/kg per d), giving half of the dose at 9:00 AM and the second half of the dose at 7:00 PM. After nimesulide administration was finished, a recovery period of 3 days was allowed. Twenty-four-hour urine samples were measured between 9:00 and 9:30 AM each day. Samples for measurement of GFR, plasma sodium concentration, and pK were drawn daily, 22 hours after the last feeding. In addition, blood and urine samples were obtained during the control period at the end of days 1, 4, and 8 of nimesulide administration; and at the end of days 1 and 3 of recovery period to analyze plasma renin activity (PRA), PAC, and the urinary excretion rates of PGE\(_2\), 6-keto-prostaglandin F\(_{1a}\) (6-keto-PGF\(_{1a}\)), thromboxane B\(_2\) (TXB\(_2\)), and 11-dehydro-TXB\(_2\).

**Group 2 (n=6)**
The experimental protocol was similar to that described for group 1, with the exception that total sodium load was increased to \( \sim 200 \) mEq/d by continuously infusing isotonic saline at a rate of 1265 mL/d. Urinary PG and thromboxane excretion, PRA, and PAC were not evaluated in this group of dogs.

**Group 3 (n=6)**
The protocol was similar to that described for group 1, with exception that only isotonic glucose (225 mL/d) was infused during the experiment. Total sodium intake was 4 to 6 mEq/d. Urinary PG and thromboxane excretion were not evaluated in this group.

**Group 4 (n=8)**
Total sodium load was 70 mEq/d. After a control period of 3 days, N\(^\circ\)-nitro-L-arginine methyl ester (L-NAME) was infused (5 μg/kg per min) during 10 days. Forty-eight hours after L-NAME infusion was started, nimesulide was administered during 8 days as in groups 1, 2, and 3. After L-NAME and nimesulide infusions were finished, a recovery period of 3 days was allowed.

**Group 5 (n=8)**
The experimental protocol was similar to that described for group 4, with the exception that total sodium load was increased to \( \sim 200 \) mEq/d by infusing isotonic saline at a rate of 1265 mL/d.

**Analytic Methods**
Sodium and potassium levels were measured by flame photometry, and GFR was determined by clearance of endogenous creatinine.\textsuperscript{10,11,15} PRA and PAC were measured using commercial RIA (DiaSorin). Urinary concentration of PGE\(_2\), 6-keto-PGF\(_{1a}\), TXB\(_2\), and 11-dehydro-TXB\(_2\) were measured using commercial EIA kits (Cayman Chemical).

**Statistical Analysis**
Data are expressed as mean±SE. Significance of differences in values of each day in the same group, with respect to the control period, was evaluated using a 1-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 2-way ANOVA and the Duncan test. \( P<0.05 \) was considered significant.

**Results**

**Group 1**
MAP had a basal value of 105±1 mm Hg and did not change during the experiment. Figure 1 illustrates the renal hemodynamic effects of nimesulide in dogs with normal sodium load. GFR did not change throughout the experiment from a control value of 42±2 mL/min, and RBF decreased (\( P<0.05 \)) during COX-2 inhibition from a basal value of 247±25 mL/min. The maximum decrease in RBF (17\%) occurred during the second day of nimesulide administration, and this fall in RBF was only significant from days 1 to 6 of COX-2 inhibition. During the first day of the recovery period, RBF increased to 252±21 mL/min and remained at the same level the following 2 days. Urinary sodium excretion (UNaV) decreased (\( P<0.05 \)) from a basal value of 72±5 to 40±5 mEq/d the first day of COX-2 inhibition and returned to control level during the following 7 days (Figure 2, top panel). The first day of recovery period, UNaV increased...
UNaV decreased \((P<0.05)\) to \(103\pm8\) mEq/d and returned to basal values on the last 2 days of experiment. As occurred with UNaV, urine flow rate decreased \((P<0.05)\) on the first day of COX-2 inhibition (from \(1110\pm138\) to \(888\pm164\) mL/d), returned to basal levels during the following 7 days, and increased transitorily \((1351\pm149\) mL/d; \(P<0.05)\) on the first day of the recovery period. As in the other 4 groups, plasma sodium concentration did not change throughout the experiment. Plasma potassium only increased transitorily the fourth and fifth days of COX-2 inhibition (Table).

PRA decreased \((P<0.05)\) to \(0.25\pm0.14\) and \(0.47\pm0.26\) ng Ang I/mL per hour during the fourth and last day of COX-2 inhibition (basal value, \(0.82\pm0.23\) ng Ang I/mL per hour) (Figure 3). During the first day of COX-2 inhibition \((0.63\pm0.42\) ng Ang I/mL per hour) and during the first \((0.76\pm0.21\) ng Ang I/mL per hour) and third \((1.0\pm0.17\) ng Ang I/mL per hour) days of the recovery period, PRA had similar values to those found in the control period. As shown in Figure 4 (top panel), PAC was not altered during COX-2 inhibition in dogs with normal sodium load (control value, \(24\pm7\) pg/mL).

Figure 5 shows that urinary PGE\textsubscript{2} excretion rate decreased by \(>55\%\) \((P<0.05)\) the first day of COX-2 inhibition (basal value, \(317\pm44\) pg/mL), remained decreased \((P<0.05)\) until the last day of nimesulide infusion, and returned to basal values during the recovery period. The response of urinary 6-keto-PGF\textsubscript{1α} excretion was similar to that of PGE\textsubscript{2} excretion. Contrary to the decrease of PGE\textsubscript{2} and 6-keto-PGF\textsubscript{1α}, no significant changes in the urinary excretion rate of TXB\textsubscript{2} (Figure 5, lower panel) and 11-dehydro-TXB\textsubscript{2} (basal values, \(244\pm35\) and \(10\pm3\) pg/min, respectively) were found in response to the prolonged COX-2 inhibition.

**Group 2**

COX-2 inhibition in dogs with high sodium load did not modify MAP (basal value, \(104\pm4\) mm Hg). GFR was not altered during COX-2 inhibition but RBF decreased by 12%
The L-NAME infusion to dogs with normal sodium intake elicited an increment of MAP (103±4 to 121±6 mm Hg; P<0.05) that was not modified during the simultaneous nimesulide administration. MAP decreased progressively when L-NAME and nimesulide administrations finished. As shown in Figure 6, GFR did not change and RBF decreased transiently during the first 2 days of NO synthesis inhibition. Contrary to the response found in group 1 (Figure 1), COX-2 inhibition in this group elicited a significant and continuous fall in GFR (by 41% on the first day of simultaneous L-NAME and nimesulide administration). The COX-2 inhibition also elicited in this group an important and continuous fall in RBF (154±6 mL/min on the last day of COX-2 and NO inhibition, versus 226±15 mL/min during control period). Both, GFR and RBF gradually returned to basal levels during recovery period (Figure 6).

UNaV did not change during the first 2 days of L-NAME infusion (basal value, 70±3 mEq/d), decreased the first day of simultaneous NO and COX-2 inhibition (30±6 mEq/d; P<0.05) and returned to control levels the following 7 days (Figure 7). UNaV increased to 118±13 mEq/d (P<0.05) on the first day of the recovery period and returned to basal levels thereafter. Urine flow rate did not change significantly during the first 2 days of NO synthesis inhibition (basal value, 978±57 mL/d), decreased only on the first day of simulta-
neous NO and COX-2 inhibition (664±63 mL/d; P<0.05), and increased transitorily the first day of the recovery period (1393±106 mL/d; P<0.05). Contrary to what was found in group 1, prolonged COX-2 inhibition in dogs with reduced NO synthesis elicited a significant and continuous elevation in pK (Table). The increment was greater (P<0.05) than that found in group 1 (Table).

Group 5
The L-NAME administration to dogs with high sodium load induced an increase in MAP (104±2 to 122±4 mm Hg; P<0.05) that was not modified during the simultaneous COX-2 and NO synthesis inhibition. MAP decreased during the recovery period. Figure 6 shows that RBF and GFR decreased (P<0.05) transitorily during the first day of L-NAME infusion. As occurred in dogs with normal sodium intake, prolonged COX-2 inhibition in this group elicited a significant and continuous decrease in GFR and RBF (Figure 6). Although there is a tendency to be greater in dogs with normal sodium, the decrease of GFR and RBF induced by the simultaneous inhibition of COX-2 and NO synthesis was similar in dogs with normal and high sodium load (Figure 6).

UNaV did not change during the first 2 days of L-NAME infusion (basal value, 213±12 mEq/d), decreased to 154±11 mEq/d (P<0.05) the first day of simultaneous inhibition of COX-2 and NO synthesis, and returned to basal levels the following 7 days (Figure 7). UNaV increased to 277±11 mEq/d (P<0.05) on the first day of the recovery period and gradually returned to basal levels thereafter. As with UNaV, urine flow rate did not change the first 2 days of L-NAME infusion (basal value, 2033±97 mL/d), decreased only on the first day of simultaneous administration of L-NAME and nimesulide (1624±115 mL/d; P<0.05), and increased the first day of the recovery period (2406±26 mL/d; P<0.05). Similar to what it was found during normal sodium intake, the nimesulide administration to dogs with high sodium load and reduced NO synthesis elicited a significant and continuous elevation in pK (Table).

Discussion
This is the first study evaluating the renal hemodynamic and excretory response to the prolonged administration of a selective COX-2 inhibitor when sodium load is low, normal, or elevated. This study also reports for the first time the changes in pK and PAC during a prolonged COX-2 inhibition. Finally, this is the first study evaluating whether COX-2-derived PG plays a more important role in the prolonged control of renal function when NO synthesis is reduced. The role of COX-2 in the regulation of renal function was assessed in conscious dogs by administering a COX-2 inhibitor (nimesulide) during 8 days. The lack of an effect on urinary excretion rate of TXB2 and 11-dehydro-TXB2 suggests that the dose of nimesulide used has no effect on COX-1 activity. By contrast, nimesulide infusion re-
sulted in a decrease of PGE2 and 6-keto-PGF1α. With these results, it may be proposed that the nimesulide effects are secondary to a reduction in PGE2 and 6-keto-PGF1α, because both PGs play an important role in regulating renal function.9,18 The absence of changes in MAP during prolonged COX-2 inhibition (even during NO synthesis inhibition) was expected because (1) COX-2 is not constitutively expressed by endothelial and vascular smooth muscle cells,19 (2) no COX-2 immunoreactivity is detected in renal arterioles,20 and (3) only minimal COX-2 expression is detected in endothelial cells of the dog aorta.21

Prolonged COX-2 inhibition in dogs with normal sodium load did not induce changes in GFR and elicited a mild decrease in RBF. When comparing the renal hemodynamic effects of a nonselective COX inhibitor10,11 with those reported in the present study, it can be proposed that the metabolites derived from both COX isoforms are involved in the regulation of renal hemodynamic. This notion is supported by results obtained in healthy adults17 and suggests the preferential use of selective COX-2 inhibitors for treatment of inflammatory processes, as opposed to nonselective inhibitors, when sodium intake is normal. However, both inhibitors may elicit similar renal hemodynamic effects when sodium intake is low because COX-2 expression increases in the renal cortex, and this expression is more than 2-fold greater than that of COX-1 when sodium intake is low.2 The fall in GFR during COX-2 inhibition in dogs with low sodium intake was expected because, in the setting of volume depletion, endogenous PG helps to maintain GFR.13 The greater renal vasoconstriction found in the present study during low sodium intake could be partly secondary to the vasoactive effects elicited by the endogenous norepinephrine levels because it is known that renal sympathetic activity is enhanced during low sodium intake22 and that COX-2–derived PGs modulate the renal vasoconstriction induced by norepinephrine.23 COX-2 may also be more important in regulating renal hemodynamic during low sodium intake because it can metabolize 20-hydroxyeicosatetraenoic acid (20-HETE) to PG analogs and therefore can reduce the vasoconstrictor effects of 20-HETE.24

It was not expected to find that prolonged COX-2 inhibition elicits a similar renal vasoconstriction during normal and high sodium load because it has been reported that COX-2 expression in the renal cortex decreases when sodium intake changes from normal to a high level.2,3 One possibility to explain the vasoconstriction induced by COX-2 inhibition during high sodium load is that COX-2 activity is enhanced as a consequence of a greater NO production. This hypothesis is based on studies proposing that NO synthesis is increased when sodium intake is elevated15,16 and that NO can enhance COX-2 activity.25,26 Nevertheless, it may be also possible that COX-2 activity in the renal cortex is similar during normal and high sodium intake.1

The sodium retention in response to the prolonged COX-2 inhibition may be explained by the facts that COX-2 is localized in cTALH, macula densa, and medullary interstitial cells1 and that PGs play an important role in regulating medullary blood flow and sodium transport by adjacent tubule epithelial cells, including the proximal tubule and collecting duct.9 In support of a proximal tubular effect of COX-2–derived PG, previous studies have shown that COX-2 inhibition induces a significant decrease in fractional lithium excretion.7,27 The transitory effect on UNaV may be explained by a change in other regulatory mechanisms that compensate the sodium retaining effects induced by COX-2 inhibition. Because COX-2 expression is enhanced in the renal medulla during high sodium intake,2,3 one unexpected result is that sodium retention was not greater in dogs with high sodium than in those with normal sodium load. One hypothesis to explain this similar sodium retention is that the changes in COX-2 activity during high sodium load may not be as important as one might expect from the changes in COX-2 protein expression.

Several studies have evaluated whether there is an interaction between NO and COX-2 in the acute regulation of renal function with conflicting results.25,26,28,29 However, it is unknown whether there is an interaction between NO and COX-2–derived PG in the long-term regulation of renal function. It has been proposed that endogenous PGs play a more important role in regulating renal function when NO synthesis is reduced,10,14 but it remained to be elucidated whether these PGs are derived from COX-1 or COX-2. When NO production was reduced in the present study, the nimesulide administration elicited a decrease in GFR that was greater than that induced by the reduction in either NO or PG synthesis. These results suggest that COX-2 contributes to the prolonged regulation of renal hemodynamic when NO synthesis is diminished, by producing vasodilator PGs that reduced the vasoconstriction induced by the decrease in NO. This notion is supported by studies reporting that NO synthesis inhibition stimulates COX-2–mediated production of vasodilatory PGs.30 The decrease in GFR and RBF found during COX-2 inhibition, when NO synthesis was reduced, is similar to the renal hemodynamic response found previously by our group during the simultaneous administration of L-NAME and a nonselective COX inhibitor.10 Taken together from the results obtained in both studies, it may be proposed that COX-2 (rather than COX-1) is involved in producing the PGs that regulate renal hemodynamic when NO synthesis is diminished. It can also be suggested that both nonselective and selective COX-2 inhibitors share similar risks for adverse renal hemodynamic effects when NO synthesis is reduced.

The prolonged administration of the COX-2 inhibitor also reduced transitorily UNaV and urine flow rate when NO synthesis was diminished, and this response was similar in dogs with normal and high sodium load. From our findings, we consider less likely that COX-2 is relevantly involved in the long-term regulation of renal excretory function, even when NO production is diminished. The fact that the prolonged administration of a nonselective COX inhibitor (in L-NAME–pretreated dogs) induced a continuous and significant decrease in UNaV10 suggests that the COX-1 isoform is involved in producing the PG that regulate renal excretory function when endogenous NO synthesis is reduced.
The fall in PRA observed during prolonged COX-2 inhibition was expected because it has been proposed that COX-2 plays an important role in mediating the rise in PRA that follows a decrease in sodium intake. However, PRA remained relatively elevated during COX-2 inhibition in our group of dogs with low sodium intake. This response may be explained by the fact that other mechanisms, besides COX-2–derived metabolites, are also involved in the regulation of renin release during low sodium intake.

An increase in pK and a decrease in PAC during administration of nonselective COX inhibitors have been described, and the hyperkalemia seems to be secondary to a fall in PAC. Despite the many studies performed evaluating the effects elicited by selective COX-2 inhibitors, it has not been reported whether COX-2 inhibition has any effect on pK and PAC. We have found that prolonged COX-2 inhibition only elicits a mild and transitory elevation in pK when sodium load is normal, and induces an important and continuous hyperkalemia in dogs with low sodium intake. However, prolonged COX-2 inhibition elevates pK independently of sodium intake when endogenous NO synthesis is reduced. It remains to be elucidated the mechanism by which COX-2 inhibition enhances pK, but it is evident from our results that the important hyperkalemia observed during low sodium intake is not secondary to a decrease in PAC. The reason is that pK increased on the first day of nimesulide administration, with a progressive increment during the following 7 days, and there was only a mild fall of PAC during the last day of COX-2 inhibition. It has been suggested that the hyperkalemia induced by COX inhibitors could be secondary to the activation of a high-conductance K+ channel described in collecting tubules. One, at least partial, explanation for the dissociation between renin and potassium and aldosterone during COX-2 inhibition is that the elevated potassium may be promoting increased adrenal aldosterone production independent of the effects of angiotensin II. Taken together, our results provide support for the hypothesis that COX-2–derived metabolites are implicated in the long-term regulation of pK but not in that of PAC when sodium intake is chronically reduced.

**Perspectives**

The results obtained in this study suggest that COX-2–derived metabolites play a minor role in the long-term control of renal hemodynamics when sodium intake is normal or high, and a major role in the long-term regulation of RBF and GFR when sodium intake is low. Another novel finding is that renal hemodynamics is much more sensitive to the prolonged administration of a selective COX-2 inhibitor when endogenous NO production is reduced. The results showing that prolonged COX-2 inhibition elicits a continuous increase in renal vascular resistance and only a transitory sodium retention suggest that COX-2–mediated synthesis is predominantly responsible for dilator prostanooids. Finally, this is the first study showing that prolonged administration of a selective COX-2 inhibitor led to a significant increase in pK that is more significant when NO synthesis is reduced, and showing that this hyperkalemia is not secondary to a decrease in PAC. Further studies are needed to evaluate whether prolonged administration of selective COX-2 inhibitors to humans elicits significant changes in renal hemodynamics when sodium intake is elevated, and also to examine whether prolonged COX-2 inhibition induced greater changes in renal hemodynamics in situations in which endogenous NO levels are diminished.

**Acknowledgments**

This study was supported by grants from the Fondo de Investigaciones Sanitarias of Spain (FIS 2001/170) and Fundación Seneca (PI-73/890/FS/01) of Murcia, Spain. F. Roig was partly supported by a grant from the FIS of Spain (FIS, 98/1309).

**References**


Role of Cyclooxygenase-2 in the Prolonged Regulation of Renal Function
Francisco Roig, María T. Llinás, Ruth López and F. Javier Salazar

Hypertension. 2002;40:721-728; originally published online September 30, 2002;
doi: 10.1161/01.HYP.0000036451.76323.29

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2002 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://hyper.ahajournals.org/content/40/5/721

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

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

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