Renal Changes Induced by a Cyclooxygenase-2 Inhibitor During Normal and Low Sodium Intake

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Abstract—Cyclooxygenase-2 (COX-2) has been identified in renal tissues under normal conditions, with its expression enhanced during sodium restriction. To evaluate the role of COX-2–derived metabolites in the regulation of renal function, we infused a selective inhibitor (nimesulide) in anesthetized dogs with normal or low sodium intake. The renal effects elicited by nimesulide and a non–isoenzyme-specific inhibitor (meclofenamate) were compared during normal sodium intake. In ex vivo assays, meclofenamate, but not nimesulide, prevented the platelet aggregation elicited by arachidonic acid. During normal sodium intake, nimesulide infusion (n=6) had no effects on arterial pressure or renal hemodynamics but did reduce urinary sodium excretion, urine flow rate, and fractional lithium excretion. In contrast, nimesulide administration increased arterial pressure and decreased renal blood flow, urine flow rate, and fractional lithium excretion during low sodium intake (n=6). COX-2 inhibition reduced urinary prostaglandin E2 excretion in both groups but did not modify plasma renin activity in dogs with low (8.1±1.1 ng angiotensin I·mL⁻¹·h⁻¹) or normal (1.8±0.4 ng angiotensin I·mL⁻¹·h⁻¹) sodium intake. Meclofenamate infusion in dogs with normal sodium intake (n=8) induced a greater renal hemodynamic effect than nimesulide infusion. These results suggest that COX-2–derived metabolites (1) are involved in the regulation of sodium excretion in dogs with normal sodium intake, (2) play an important role in the regulation of renal hemodynamic and excretory function in dogs with low sodium intake, and (3) are not involved in the maintenance of the high renin levels during a long-term decrease in sodium intake. (Hypertension. 2000;36:276-281.)

Key Words: renal circulation ■ kidney ■ cyclooxygenase ■ sodium ■ renin ■ prostaglandins

The role of cyclooxygenase-derived metabolites in the regulation of renal hemodynamic and excretory function has been demonstrated in many studies.1–6 Two different cyclooxygenase (COX) isoforms have been identified: COX-1 is present at relatively stable levels in many tissues, and COX-2 is induced by several growth factors and inflammatory mediators.7 COX-2 is also constitutively expressed in the kidney8 and other tissues.9 Renal COX-2 expression has been observed in macula densa,8 glomeruli and vasa recta,10 loop of Henle,11 and medullary interstitial cells.12 It has also been shown that renal COX-2 expression is stimulated in situations, such as a long-term decrease in sodium intake13 and renovascular hypertension,14 in which renin release is chronically enhanced. However, the role of COX-2–derived metabolites is not well defined in the regulation of renal function during normal sodium intake, and whether this role is enhanced after a long-term reduction in sodium intake is not determined. The first objective of the present study was to evaluate the role of COX-2–derived metabolites in the regulation of renal function in dogs with normal or low sodium intake. We examined the role of the COX-2–derived metabolites by infusing an arylsulfonamide (nimesulide) that inhibits the COX-2 isoform with high selectivity.15–17 We also evaluated whether the enhanced renin activity, induced by low sodium intake, is modified by acute COX-2 inhibition. To accomplish these objectives, nimesulide was administered at 2 doses, with the lowest similar to that used for inflammation treatment in humans.18 The last objective of this study was to compare the renal changes elicited by nimesulide with those induced by a non–isoenzyme-specific COX inhibitor (meclofenamate).

The effect of nimesulide on COX-1 activity was examined in ex vivo assays in which arachidonic acid–induced platelet aggregation was evaluated in platelet-rich plasma (PRP) obtained from blood samples withdrawn from dogs treated with nimesulide or meclofenamate. It is expected that this platelet aggregation is impaired in meclofenamate-treated dogs and not significantly altered in nimesulide-treated dogs, because the thromboxane A2 accounting for platelet aggregation is COX-1 dependent.19 Platelet aggregation was also tested with in vitro assays.

Methods

Experiments were performed in mongrel dogs of either sex (17 to 25 kg) with free access to tap water. Protocols were designed according to the guiding principles approved by the Council of the American

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Physiological Society. Six days before the experiments, dogs were housed in individual metabolic cages and fed low- or normal-sodium diet. On the fifth and sixth days before the experiments, diuretics were infused in the dogs on a sodium-deficient diet (Hill Pet Products). Diuretics were administered to ensure the dogs were volume depleted.20 A low-sodium diet provided 5 to 7 mmol sodium/day.

Surgical preparation was performed in anesthetized dogs (30 mg/kg sodium pentobarbital IV) as previously described.6,20,21 Catheters were placed in the femoral artery for measurement of mean arterial pressure (MAP) and in the femoral vein for infusion of insulin and nimesulide or meclofenamate. Insulin was dissolved in isotonic saline (0.9% NaCl) for experiments performed in dogs with normal sodium intake and dissolved in a glucose solution (5%) for experiments performed in dogs with low sodium intake. The renal arteries were fitted with noncannulating electromagnetic flow probes and connected to flowmeters. A 45-minute stabilization period was allowed before experimental maneuvers were begun.

Experimental Groups

Group 1

The effect of nimesulide and meclofenamate on COX-1 activity was examined in group 1 (n=8) through evaluation of the aggregation response of PRP to arachidonic acid (0.65 mmol/L). The aggregation response of PRP was examined essentially as previously described22 in blood samples withdrawn during the control period (predrug) and 45 minutes after the start of the intravenous infusion of meclofenamate (n=4) or nimesulide (n=4). Meclofenamate was continuously administered (10 μg·kg⁻¹·min⁻¹). Nimesulide was infused as a bolus (0.75 mg/kg) and continuously (5 μg·kg⁻¹·min⁻¹).

Group 2

The protocol was performed in dogs with a normal sodium intake (n=6). The evening before the experiment, LiCO₃ (800 mg) was administered orally. After two 15-minute control clearance periods, nimesulide was intravenously infused as a bolus (0.75 mg/kg) and continuously during 75 minutes (5 μg·kg⁻¹·min⁻¹). Forty-five minutes after initiation of the continuous nimesulide infusion, 2 additional 15-minute clearances were obtained. Then, a second dose of nimesulide was administered as a bolus (1.5 mg/kg) and a continuous infusion rate of 10 μg·kg⁻¹·min⁻¹. Forty-five minutes after initiation of this nimesulide infusion, 2 additional 15-minute clearances were obtained.

Group 3

The experimental protocol in this group (n=6) was similar to that accomplished in group 2, with the only difference that it was performed in dogs with a low sodium intake.

Group 4

The protocol was performed in dogs with normal sodium intake (n=8). After two 15-minute control clearance periods, meclofenamate was infused during 75 minutes (10 μg·kg⁻¹·min⁻¹). Forty-five minutes after initiation of meclofenamate infusion, 2 additional 15-minute clearances were obtained. The meclofenamate dose used has been reported to decrease the urinary excretion of prostaglandin E₂ (PGE₂) by 90%.23

Analytical Methods

Renal clearances were undertaken during each experimental period to determine glomerular filtration rate (GFR); sodium, potassium, and lithium excretions; urine flow rate (UV); urine osmolality; and urinary PGE₂ excretion rate. Blood samples for plasma renin activity (PRA), hematocrit, plasma osmolality, and plasma sodium, potassium, lithium, and inulin concentrations were also obtained. Inulin concentrations were analyzed with the anthrone method. Concentrations of sodium and potassium were measured with flame photometry. Proximal tubule sodium reabsorption was estimated with the lithium clearance technique. Lithium concentrations were measured with flame emission spectrophotometry (model 5500; Perkin–Elmer).

Results

Group 1

Ex Vivo Assays

The aggregation in PRP observed in samples withdrawn during the basal period (predrug) (69±8%) was completely blocked in each PRP obtained from blood collected after treatment with meclofenamate. The platelet aggregation was not altered in PRP obtained from blood withdrawn after nimesulide infusion (63±12%).

In Vitro Assays

Figure 1 shows the aggregation response of PRP samples preincubated with different doses of meclofenamate or nimesulide, represented as a percent of the response found in the PRP preincubated with vehicle (0.9% NaCl). As shown,
by 29±17%, although this reduction did not reach statistical significance. In addition, aggregation was negligible in PRP preincubated with meclofenamate at 5 or 10 μmol/L. Figure 1 shows that meclofenamate is more effective than nimesulide in blocking the platelet response to arachidonic acid. It can be observed that aggregation was not significantly modified by preincubation of the PRP with nimesulide at 10, 20, or 50 μmol/L. At a dose of 100 μmol/L, nimesulide reduced by 69±22% the aggregation observed in vehicle-pretreated PRP. However, this reduction was not significant because of a great variability in the response of PRP samples preincubated with such a high dose of nimesulide.

Group 2

Figure 2 shows that intravenous infusion of the 2 nimesulide doses did not elicit changes in MAP, GFR, and renal blood flow (RBF) in dogs with normal sodium intake. However, nimesulide induced a significant decrease (P<0.05) in urinary sodium excretion (UNaV), UV, and fractional lithium excretion (FeLi). It can be observed in Figure 3 that the lowest dose of nimesulide led to a decrease (P<0.05) in UNaV (64±10 to 32±8 μmol/min), UV (0.21±0.02 to 0.10±0.01 mL/min), and FeLi (41±3% to 29±4%). Administration of the largest dose of nimesulide did not induce a further decrease in UNaV (27±7 μmol/min), UV (0.12±0.02 mL/min), and FeLi (24±2%) (Figure 3).

Nimesulide did not modify PRA from a basal value of 1.8±0.4 ng angiotensin (Ang) I·mL⁻¹·h⁻¹ but led to a 40% decrease (P<0.05) in urinary PGE₂ excretion (5.8±0.6 to 3.5±0.3 ng/min) (Figure 4). No significant changes in plasma osmolality and plasma sodium and potassium concentrations were observed throughout the experiment. Urinary osmolality increased (P<0.05) with the nimesulide infusion.

Group 3

Contrary to the results found in dogs with a normal sodium intake, COX-2 inhibition in this group led to a 10% increase in MAP and a 20% fall in RBF (P<0.05) (Figure 2). The greatest dose of nimesulide also induced a significant decrease in GFR (32±1 to 25±2 mL/min, P<0.05). UNaV did not change during nimesulide infusion (basal value 3.0±0.8 μmol/min) (Figure 3). However, decreases in UV (0.12±0.01 to 0.06±0.01 mL/min) and FeLi (19.4±2.2% to 7.8±1.2%) were induced by COX-2 inhibition with the lowest dose of nimesulide. These changes in UV and FeLi were not enhanced by the greatest dose (Figure 3).

As expected, basal PRA was enhanced (8.1±1.1 ng Ang I·mL⁻¹·h⁻¹) in dogs with low sodium intake (Figure 4). However, these PRA levels were not modified by COX-2 inhibition. It can be observed in Figure 4 that nimesulide infusion reduced (P<0.05) urinary PGE₂ excretion from a basal value of 3.7±0.5 to 1.7±0.3 ng/min. Urinary PGE₂ excretion, after nimesulide, was lower (P<0.05) in dogs fed a low-sodium diet than in those with normal sodium intake. No changes in plasma osmolality and plasma sodium concentration were found throughout the experiment. Plasma potassium levels were slightly elevated after infusion of the greatest dose of nimesulide (2.7±0.2 to 3.0±0.2 mmol/L, P<0.05). Urine osmolality increased (P<0.05) with nimesulide infusion.

Group 4

Figure 5 shows the effects elicited by meclofenamate in dogs with normal sodium intake. It can be observed that this nonspecific COX inhibitor induced an increase in MAP (129±4 to 138±6 mm Hg, P<0.05) and a decrease in RBF

Figure 2. Changes in MAP, GFR, and RBF in dogs with a normal or low sodium intake in response to the intravenous administration of 2 nimesulide doses (dose 1, 0.75 mg/kg bolus plus infusion of 5 μg·kg⁻¹·min⁻¹; dose 2, 1.5 mg/kg bolus plus 10 μg·kg⁻¹·min⁻¹). *P<0.05 vs baseline.

Figure 3. Changes in UNaV, UV, and FeLi in dogs with a normal or low sodium intake in response to the intravenous administration of 2 nimesulide doses (dose 1, 0.75 mg/kg bolus plus infusion of 5 μg·kg⁻¹·min⁻¹; dose 2, 1.5 mg/kg bolus plus 10 μg·kg⁻¹·min⁻¹). *P<0.05 vs baseline.
In contrast to the lack of changes in renal vascular resistance (RVR) during nimesulide infusion in dogs with normal sodium intake, meclofenamate elicited a 27% elevation in RVR (from 0.77 ± 0.06 to 0.98 ± 0.08 mm Hg·mL⁻¹·min⁻¹). Meclofenamate also led to a decrease (P < 0.05) in UNaV (70 ± 11 to 22 ± 3 mmol/min) and UV (0.46 ± 0.08 to 0.11 ± 0.02 mL/min) (Figure 5) and an increase (P < 0.05) in urinary osmolality. No changes were found in plasma osmolality and plasma sodium and potassium concentrations.

Discussion

The results of this study suggest that COX-2–derived metabolites are not involved in the regulation of renal hemodynamic when sodium intake is normal. The lack of a renal hemodynamic change in response to nimesulide is supported by the fact that COX-2 expression is low in the vasculature of renal cortex during normal sodium intake.8,26 The renal vasoconstriction induced by meclofenamate was expected because COX-1 is abundant in the renal vasculature.10 The different renal hemodynamic responses to nimesulide and meclofenamate is supported by the fact that...
nimesulide reduces urinary PGE₂ excretion to a lower extent (≈40%) than does meclofenamate (≈90%).

Changes in renal excretory function in dogs with normal sodium during COX-2 inhibition can be explained by results that show this isozyme is constitutively expressed in several tubular segments, vasa recta, and medullary interstitial cells. Previous studies have reported evidence of the role of PGE₂ in the regulation of both medullary blood flow (MBF) and sodium reabsorption in the loop of Henle and collecting tubule. Although the difference is not significant, the decrement in sodium excretion in response to the non–isozyme-specific COX inhibitor (48±11 μmol/min) tended to be greater than that induced by the COX-2 inhibitor (33±9 μmol/min). Meclofenamate seems to be more effective than nimesulide in decreasing sodium excretion, because MAP increased with meclofenamate and did not change with nimesulide. The increase in MAP in meclofenamate-treated dogs could mask the effect of this drug on sodium excretion. In support of the hypothesis that a nonselective COX inhibitor reduces the renal excretory ability to a greater extent than nimesulide, we have demonstrated that meclofenamate, but not nimesulide, abolishes the bradykinin-induced natriuresis and diuresis in Nω-nitro-L-arginine methyl ester–treated dogs.

The nimesulide effects on UNaV and UV seem to be secondary, at least in part, to an increased proximal tubule reabsorption, because a significant decrease in FeLi was found. This change in FeLi seems to be an indirect effect because COX-2 expression has not been detected in the proximal tubule. A small number of EP₄ receptors have been found in this tubular segment, but the activation of these receptors induces only renal hemodynamic changes. One possible explanation for the increase in proximal reabsorption secondary to increments in RIHP is abolished by prostaglandin synthesis inhibition.

The results obtained in the present study suggest that COX-2–derived metabolites play an important role in the regulation of arterial pressure and renal hemodynamics when sodium intake is chronically reduced. The fact that COX-2 expression in the renal cortex is significantly enhanced during salt restriction supports this concept. The nimesulide-induced systemic and renal vasoconstriction could be secondary not only to the reduction in prostaglandin levels but also to the effects induced by the endogenous vasoconstrictors. COX-2–derived metabolites possibly counteract the prohypertensive actions of the increased renin-angiotensin system. It also must be considered that after nimesulide administration, prostaglandin levels were lower in dogs fed a low-sodium diet than in those with normal sodium intake. These lower prostaglandin levels could contribute to the vasoconstriction observed during low sodium intake. The decrease in UV induced by COX-2 inhibition in dogs with a low sodium intake can be explained by increased water reabsorption in proximal and distal tubules.

Although COX-2 expression is upregulated in the renal cortex when sodium intake is low, basal urinary PGE₂ excretion is reduced (3.7±0.5 ng/min) with respect to PGE₂ excretion in dogs with normal sodium intake (5.8±0.6 ng/min). These results are similar to those reported previously and were expected because (1) COX-1 and COX-2 expressions in renal medulla are significantly decreased after a prolonged reduction in sodium intake and (2) PGE₂ synthesis is much greater in renal medulla than in renal cortex.

The role of COX-2–derived metabolites in the mediation of the prolonged elevation in renin release has been suggested to be important in renovascular hypertensive rats and during sodium restriction in mice. In our study, the infusion of nimesulide during 150 minutes did not modify PRA in dogs with normal or low sodium intake. One possible explanation for the lack of a decrease in PRA after nimesulide infusion is that acute changes do not necessarily reflect alterations in COX-2–mediated renin expression. It remains to be elucidated whether a chronic nimesulide infusion reduces PRA in dogs. However, we believe that we should have found a decrease in PRA if COX-2 inhibition reduces renin release in dogs, because PRA was measured in our study 75 and 150 minutes after nimesulide infusion was started. It is known that PRA decreases in response to an acute volume expansion and even to acute intrarenal atrial natriuretic peptide infusion. Our results suggest that COX-2–derived metabolites are not important for the maintenance of high renin levels when sodium intake is chronically reduced in dogs. In support of our hypothesis, Blasingham and Nasjletti found that the infusion of a non–isozyme-specific COX inhibitor does not elicit changes in PRA in dogs with low sodium intake.

In summary, the results obtained in the present study propose that the administration of a specific COX-2 inhibitor to dogs with normal sodium intake reduces renal excretory function without affecting renal hemodynamics. The decrease in sodium and water excretion seems to be secondary, at least in part, to an indirect effect on the proximal tubule. It is also suggested that the role of COX-2–derived metabolites in the regulation of renal hemodynamics is enhanced when sodium intake is reduced. The results obtained may have important clinical implications because many patients may use the new COX-2 inhibitors for the treatment of inflammatory diseases. Our study reports that specific COX-2 inhibition (1) has lower renal effects than non–isozyme-specific inhibition when sodium intake is normal (2) and may induce a renal vasoconstriction and an increase in arterial pressure when sodium intake is chronically reduced.

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