Natriuretic Response to Increased Pressure Is Preserved With COX-2 Inhibitors

Jennifer M. Gross, Jennifer E. Dwyer, Franklyn G. Knox

Abstract—Elevation of renal interstitial hydrostatic pressure (RIHP) by direct renal interstitial volume expansion increases sodium excretion. This natriuretic response is blunted by the nonspecific inhibition of the cyclooxygenase (COX) enzymes. The present study tested the hypothesis that the natriuretic response to increased RIHP during direct renal interstitial volume expansion is dependent on COX-1 but not COX-2. RIHP and fractional excretion of sodium (FE Na ) were measured before and after direct renal interstitial volume expansion in control rats (n = 7), rats infused with the COX-1 inhibitor piroxicam (n = 6, 1.5 mg/kg), and rats infused with the COX-2 inhibitors NS-398 (n = 5, 1.5 mg/kg) and meloxicam (n = 6, 0.3 mg/kg). In control animals, direct renal interstitial volume expansion significantly increased RIHP (Δ2.3 ± 0.5 mm Hg, P < 0.05) and FE Na (Δ1.1 ± 0.3%, P < 0.05). Likewise, in animals infused with NS-398 or meloxicam, direct renal interstitial volume expansion significantly increased RIHP (Δ1.8 ± 0.6 mm Hg, P < 0.05, and Δ1.7 ± 0.3 mm Hg, P < 0.05) and FE Na (Δ1.5 ± 0.4%, P < 0.05, and Δ1.1 ± 0.3%, P < 0.05), respectively. In contrast, infusion of piroxicam significantly blunted the natriuretic response to direct renal interstitial volume expansion (ΔFE Na 0.3% ± 0.2%), even though RIHP was increased (Δ1.9 ± 0.6 mm Hg, P < 0.05). Infusion of piroxicam but not NS-398 or meloxicam blunted the natriuretic response to increased renal interstitial hydrostatic pressure, suggesting that the natriuretic response to increased blood pressure may be preserved during inhibition of COX-2. (Hypertension. 1999;34:1163-1167.)

Key Words: sodium ■ prostaglandins ■ kidney

Increases in arterial blood pressure are associated with elevations in renal interstitial hydrostatic pressure (RIHP) and fractional sodium excretion (FE Na ). When the renal capsule is removed, renal interstitial hydrostatic pressure is prevented from rising and the natriuretic response to increased blood pressure is blunted, which suggests that RIHP is an important factor linking arterial blood pressure to tubular reabsorption of sodium. The elevation in renal interstitial hydrostatic pressure in response to increased renal perfusion pressure is mimicked by direct renal interstitial volume expansion (DRIVE), the technique used in the present study. DRIVE was previously shown to significantly increase renal interstitial hydrostatic pressure and sodium excretion without affecting renal blood flow or glomerular filtration rate (GFR). The natriuretic response to increased renal perfusion pressure or direct renal interstitial volume expansion is blunted during inhibition of prostaglandin synthesis by the nonspecific cyclooxygenase inhibitors indomethacin or meclofenamate, demonstrating that the presence of prostaglandins are required for the full expression of the natriuretic response to increased renal interstitial hydrostatic pressure.

Nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit the activity of cyclooxygenase (COX), the enzyme responsible for the conversion of arachidonic acid to prostanoids. Two separate isoforms of this enzyme have been identified, COX-1 and COX-2. Because inflammatory mediators and growth factors induce COX-2 expression but not COX-1 expression, COX-2 has been generally described as the more important COX isoform in mediating inflammation, whereas COX-1 is thought to be involved with the physiological homeostasis regulation of renal, gastrointestinal, and platelet functions. However, recent studies have demonstrated constitutive COX-2 expression in the kidney and its dependence on volume status. Thus the role of COX-2-synthesized prostaglandins in the kidney is not clear.

NS-398 and meloxicam are NSAIDs that have been shown to have potent anti-inflammatory activity in the rat. NS-398 has been demonstrated to selectively inhibit COX-2 without affecting COX-1 activity. Likewise, meloxicam has consistently demonstrated a preference to inhibit COX-2 with a COX-2/COX-1 IC50 ratio of 0.33. Conversely, piroxicam is a NSAID that has demonstrated a preference to inhibit COX-1 (IC50 ratio of 250). Traditional NSAIDs such...
as indomethacin are nonselective COX inhibitors (IC50 ratio of 60).19

The present study compared the effects of therapeutically similar anti-inflammatory doses of piroxicam, NS-398, or meloxicam on the natriuretic response to increased renal interstitial hydrostatic pressure. We hypothesized that piroxicam infusion would blunt the natriuretic response to increased renal interstitial hydrostatic pressure induced by DRIVE, whereas NS-398 and meloxicam infusion would have no effect on the natriuretic response.

Methods

At least 2 weeks before the acute experiment, polyethylene matrices were implanted in the left kidney of 250-g male Sprague-Dawley rats as previously described.2 Briefly, the rats were anesthetized with an intramuscular injection of equal volumes of Xylazine (Lloyd Laboratories) and Ketalar (Parke-Davis) (100 mg/kg body wt). Under aseptic conditions, the rats were placed on a heated table and a midline abdominal incision was made. The left kidney was exposed and 2 polyethylene matrices were implanted in each renal pole. The muscle layer was closed with a continuous 3-0 vicryl suture, and the skin layer was closed with separate silk 4-0 sutures.

On the day of the acute experiment, rats with implanted matrices were anesthetized with an intraperitoneal injection (100 mg/kg body wt) of Inactin (Byk-Gulden) and were placed on a heated table to maintain rectal temperature at ∼37°C. After tracheostomy, a polyethylene (PE-50) catheter was inserted in the jugular veins for infusions and in the carotid artery for arterial blood sampling and the monitoring of mean arterial pressure (MAP). Intravenous infusions of 0.9% NaCl and 2% inulin in 6 mmol/L LiCl dissolved in 0.9% NaCl were initiated each at 1 mL/100 g body wt per hour, and a 2-hour recovery period was allowed. Then, the implanted matrices were exposed; one was connected to a pressure transducer to continuously monitor renal interstitial hydrostatic pressure and the other matrix was used for DRIVE. A PE-50 catheter was placed in the ureter for urine collection. In the vehicle, piroxicam, and meloxicam protocols (groups 1, 2, 4, 5, 6, and 8), urine samples were collected on ice and frozen at −20°C for later determination of urinary PGE2. In all protocols, a blood sample was collected for determination of renal interstitial hydrostatic pressure.

Inhibition by Piroxicam (n = 5)

This protocol is identical to group 2 except that the COX-2 inhibitor NS-398 (1.5 mg/kg) was administered intravenously as a bolus 90 minutes after the infusions had been started. This dose of NS-398 was previously shown to completely inhibit COX-2 activity in an in vivo inflammation model without affecting COX-1 activity.15

Inhibition by Meloxicam (n = 6).

This protocol is identical to group 2 except that the COX-2 inhibitor meloxicam (0.3 mg/kg) was administered intravenously as a bolus 90 minutes after the infusions had been started. A similar dose of meloxicam (daily oral dose range from 0.10 to 0.14 mg/kg, ID50 0.12 mg/kg) was previously shown to dose-dependently inhibit paw swelling in the rat in a model of adjuvant-induced arthritis.16

Saline Time Control (n = 4)

This protocol is a time control for group 1. Two hours after initiation of intravenous infusions, a 30-minute clearance was taken. Then, a final 30-minute clearance was taken.

Piroxicam Time Control (n = 6)

This protocol is a time control for group 2. Ninety minutes after initiation of the intravenous infusions, the COX-1 inhibitor piroxicam (1.5 mg/kg bolus) was administered intravenously. After a 30-minute equilibration period, a 30-minute control clearance was taken. Then, a final 30-minute clearance was taken.

NS-398 Time Control (n = 5)

This protocol is a time control for group 4. Ninety minutes after initiation of the intravenous infusions, the COX-2 inhibitor NS-398 (1.5 mg/kg bolus) was administered intravenously. After a 30-minute equilibration period, a 30-minute control clearance was taken. Then, a final 30-minute clearance was collected.

Meloxicam Time Control (n = 7)

This protocol is a time control for group 3. Ninety minutes after initiation of the intravenous infusions, the COX-2 inhibitor meloxicam (0.3 mg/kg bolus) was administered intravenously. After a 30-minute equilibration period, a 30-minute control clearance was taken. Then, a final 30-minute clearance was collected.

Analytic Procedures

GFR was determined from the clearance of inulin. Inulin concentrations in plasma and urine were determined by the anthrone method.20 Sodium and lithium concentrations in plasma and urine were measured by flame photometry (Instrumentation Laboratory Inc). Urinary PGE2 concentrations were determined by a Prostaglandin E2 Immunoassay Kit (Cayman Chemical).

Results

 The renal responses to DRIVE in the presence and absence of piroxicam, NS-398, or meloxicam are shown in Table 1. Volume expansion of the renal interstitium by injection of 100 μL of a 2.5% albumin solution into the chronically implanted matrix significantly increased renal interstitial hydrostatic pressure from 4.4 ± 1.0 to 6.8 ± 1.3 mm Hg (Δ 2.3 ± 0.5 mm Hg, P < 0.05) in vehicle-infused rats, 4.5 ± 0.8 to 6.4 ± 1.3 mm Hg (Δ 1.9 ± 0.6 mm Hg, P < 0.05) in piroxicam-infused rats, 5.7 ± 0.8 to 7.5 ± 0.8 mm Hg (Δ 1.8 ± 0.6 mm Hg, P < 0.05) in NS-398–infused rats, and from 4.3 ± 0.9 to 6.4 ± 1.0 mm Hg (Δ 2.1 ± 0.7 mm Hg, P < 0.05) in meloxicam–infused rats.
6.0 ± 1.1 mm Hg (Δ1.7 ± 0.3 mm Hg, *P* < 0.05) in meloxicam-infused rats. The increase in RIHP was similar between these 4 groups. These increases in RIHP were associated with significant increases in FE$_{Li}$ and urinary flow rate (UV) in the vehicle, NS-398–infused, and meloxicam-infused rats. In contrast, the natriuretic response to DRIVE during piroxicam vehicle, NS-398–infused, and meloxicam-infused rats. In all time control rats after DRIVE, although this increase did not reach statistical significance (*P* = 0.07). FE$_{Li}$ significantly increased in NS-398–infused and meloxicam-infused rats in response to DRIVE. In the piroxicam-infused animals, UV and FE$_{Li}$ did not increase in response to DRIVE. GFR and MAP remained unaltered in all groups before and after DRIVE.

RIHP was stable in the vehicle time control group (7.1 ± 1.4 to 6.6 ± 1.4 mm Hg, Δ = 0.5 ± 0.4 mm Hg) and in the NS-398–infused time control rats (6.3 ± 0.5 to 6.1 ± 0.7 mm Hg, Δ = 0.2 ± 0.3 mm Hg). RIHP was measured in 6 meloxicam-infused time control rats (5.1 ± 0.8 to 5.4 ± 0.7 mm Hg, Δ0.3 ± 0.2 mm Hg) and 3 piroxicam-infused time control rats (data: 8.1 to 6.8 mm Hg, 7.3 to 5.1 mm Hg, and 4.7 to 5.0 mm Hg), and no significant changes in RIHP were observed. In the vehicle time controls and piroxicam-infused time control rats, no significant changes in FE$_{Na}$, FE$_{Li}$, or UV were observed (Table 2). However, in NS-398–infused time control rats, FE$_{Na}$ and FE$_{Li}$ significantly increased, whereas UV remained stable. Similarly, in the meloxicam-infused time control rats, FE$_{Na}$ significantly increased whereas FE$_{Li}$ and UV remained stable. In all time control rats, MAP and GFR remained stable.

PGE$_2$ excretion is presented as the combined data of each experimental group and its respective time control during the

### TABLE 2. Effects of Time and Saline Infusion on Clearance Parameters in the Presence and Absence of Cyclooxygenase-1 Inhibition by Piroxicam and Cyclooxygenase-2 Inhibition by NS-398 and Meloxicam

<table>
<thead>
<tr>
<th>Group</th>
<th>Wt, g</th>
<th>FE$_{Na}$, %</th>
<th>FE$_{Li}$, %</th>
<th>UV, μL/min</th>
<th>GFR, mL/min</th>
<th>MAP, mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle (n=4)</td>
<td>369±6</td>
<td>1.58±0.44</td>
<td>22±5</td>
<td>24±9</td>
<td>1.3±0.3</td>
<td>140±7</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>1.70±0.28</td>
<td>21±5</td>
<td>24±7</td>
<td>1.4±0.3</td>
<td>134±8</td>
</tr>
<tr>
<td>Piroxicam (n=6)</td>
<td>384±20</td>
<td>0.93±0.32</td>
<td>19±3</td>
<td>14±2</td>
<td>1.6±0.1</td>
<td>131±8</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>0.81±0.36</td>
<td>20±4</td>
<td>13±3</td>
<td>1.4±0.2</td>
<td>131±7</td>
</tr>
<tr>
<td>Vehicle</td>
<td></td>
<td>1.15±0.33*</td>
<td>28±5</td>
<td>22±5</td>
<td>2.1±0.2</td>
<td>133±6</td>
</tr>
<tr>
<td>Meloxicam (n=7)</td>
<td>389±9</td>
<td>0.68±0.21</td>
<td>22±3</td>
<td>18±4</td>
<td>2.1±0.2</td>
<td>133±4</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>1.15±0.33*</td>
<td>28±5</td>
<td>22±5</td>
<td>2.1±0.2</td>
<td>133±6</td>
</tr>
<tr>
<td>Vehicle</td>
<td></td>
<td>0.71±0.14</td>
<td>19±3</td>
<td>25±6</td>
<td>1.4±0.2</td>
<td>129±4</td>
</tr>
<tr>
<td>NS-398 (n=5)</td>
<td>356±19</td>
<td>1.04±0.18*</td>
<td>24±3*</td>
<td>28±7</td>
<td>1.2±0.1</td>
<td>129±5</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.

*Paired *t* test between the first and second clearance periods, *P* < 0.05.
first clearance period (i.e., the PGE2 excretions of groups 1 and 5, groups 2 and 6, and groups 4 and 8 were combined). Intravenous infusion of piroxicam significantly reduced urinary PGE2 excretion (7±2 mg/min) as compared with control rats (50±8 mg/min, P<0.05) and as compared with rats infused with meloxicam (36±8 mg/min, P<0.05). PGE2 excretion was not significantly different between control rats and rats infused with meloxicam P>0.05.

Discussion

The present study demonstrates that the natriuretic response to increased renal interstitial hydrostatic pressure induced by direct renal interstitial volume expansion is preserved in the presence of the COX-2 inhibitors NS-398 and meloxicam. In contrast, infusion of the COX-1 inhibitor piroxicam significantly blunted the natriuretic response to increased renal interstitial hydrostatic pressure. These studies suggest that the presence of COX-1 and not COX-2 is required for the full expression of the natriuretic response to increased renal interstitial hydrostatic pressure during direct renal interstitial volume expansion.

Prostaglandins have long been recognized as playing a role in the regulation of sodium balance during elevations in renal perfusion pressure and renal interstitial hydrostatic pressure. Carmines et al10 demonstrated in the dog that elevations in renal perfusion pressure were associated with enhanced sodium excretion. After indomethacin administration, there was a marked attenuation of the effect of increased renal perfusion pressure on sodium excretion. The natriuretic response to increased renal perfusion pressure was attenuated in rats given meclofenamate as compared with control animals.11 Likewise, the natriuretic response to increased renal interstitial hydrostatic pressure induced by DRIVE is blunted during nonselective cyclooxygenase inhibition by indomethacin or meclofenamate.7,8 Similarly, in the present study, the natriuretic response to increased renal interstitial hydrostatic pressure induced by DRIVE was significantly blunted during infusion of piroxicam, a COX-1 inhibitor, even though RIHP was significantly increased.

Sodium reabsorption in the proximal tubule decreases after increases in renal perfusion pressure.22,23 Increases in renal interstitial hydrostatic pressure induced by direct renal interstitial volume expansion inhibits proximal tubular sodium reabsorption, and this inhibition of sodium reabsorption is abolished by pretreatment with indomethacin or meclofenamate.24 Consistent with these observations, in the present study, fractional excretion of lithium, a marker for proximal tubular sodium reabsorption, significantly increased after DRIVE in rats infused with the COX-2 inhibitors NS-398 and meloxicam. On the other hand, infusion of piroxicam blocked the increase in FE(Na) after DRIVE. Taken together, these results suggest that the increase in lithium excretion after renal interstitial volume expansion reflects decreased proximal tubular sodium reabsorption and is mediated by COX-1-synthesized renal prostaglandins.

Urinary PGE2 was markedly decreased in rats infused with piroxicam. In contrast, PGE2 excretion was not significantly inhibited in rats infused with meloxicam, which is in agreement with a previous study performed in humans.25 In a study by Gonzalez-Campoy et al.26 inhibition of prostaglandin synthesis by indomethacin blunted the pressure natriuretic response to increased perfusion pressure in the dog. Subsequent infusion of PGE2 into the renal artery of the indomethacin-treated dogs completely restored the natriuretic effect of increased perfusion pressure. Because a fixed level of intrarenal PGE2 was administered, it was concluded that the presence of PGE2 was necessary for full expression of the natriuretic response to increased renal interstitial hydrostatic pressure during increased renal perfusion pressure. Likewise, in the present study the presence of prostaglandins in the control rats, the NS-398-infused rats, and meloxicam-infused rats was sufficient to allow for the full expression of the natriuretic response to increased renal interstitial hydrostatic pressure induced by DRIVE. In contrast, piroxicam infusion resulted in markedly reduced prostaglandin excretions and a blunted natriuretic response to increased RIHP as compared with control and meloxicam-infused rats.

Although NSAIDs are therapeutically effective for the conditions for which they are prescribed, adverse renal effects such as sodium retention, edema, and decreases in renal blood flow and GFR have been attributed to NSAIDs.27 Moreover, the use of NSAIDs increases the risk for initiation of antihypertensive therapy.28 It has been suggested that the selective inhibition of COX-2 would offer the anti-inflammatory therapeutic benefits associated with the use of NSAIDs and would avoid the renal sodium retention associated with these drugs.29 Although the present study did not independently show that NS-398 and meloxicam inhibited COX-2 in the kidney, these studies suggest that the inhibition of the COX-2 enzyme may preserve the pressure natriuretic response to increased blood pressure while offering anti-inflammatory relief because the natriuretic response to DRIVE was preserved in the presence of 2 separate COX-2 inhibitors at doses that have been shown to inhibit inflammation in vivo.15,16

Infusion of NS-398 and meloxicam significantly increased FE(Na) in the absence of DRIVE. It is important to note that constitutive renal COX-2 expression in the rat appears to be highly localized to the macula densa, whereas COX-1 expression is more widely distributed.12,13 The increase in sodium excretion after COX-2 inhibition in the present time-control studies might be related to decreased renin activity, because the elevation in plasma renin activity in response to salt restriction has been reported to be blocked by a COX-2 inhibitor.30

In conclusion, piroxicam but not NS-398 or meloxicam blunts the natriuretic response to increased renal interstitial hydrostatic pressure during direct renal interstitial volume expansion. These observations suggest that the natriuretic response to increased renal interstitial hydrostatic pressure may be preserved during inhibition of cyclooxygenase-2.

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