Abstract—Stretching of the renal pelvic wall activates renal mechanosensitive neurons, resulting in an increase in afferent renal nerve activity (ARNA). Prostaglandin (PG)E₂ plays a crucial role in the activation of renal mechanosensitive neurons through facilitation of the release of substance P from the sensory neurons in the renal pelvic wall. Because wall stretch may induce cyclooxygenase-2 activity, we examined whether cyclooxygenase-2 was expressed in the renal pelvic wall and whether activation of cyclooxygenase-2 contributed to the ARNA response produced through increased renal pelvic pressure. In situ hybridization showed a strong cyclooxygenase-2 mRNA signal in the papilla and subepithelial layer of the renal pelvic wall from time control kidneys and from kidneys exposed to 15 minutes of increased renal pelvic pressure in anesthetized surgically operated rats. In anesthetized rats, an increase in renal pelvic pressure increased ARNA by 40±2% and increased renal pelvic release of PGE₂ from 289±46 to 1379±182 pg/min (P<0.01). Renal pelvic perfusion with the cyclooxygenase-2 inhibitor etodolac reduced the increases in ARNA and PGE₂ by 66±7% and 55±13%, respectively (P<0.01). Likewise, the cyclooxygenase-2 inhibitor 5,5-dimethyl-3-(3-fluorophenyl)-4-(4-methylsulfonyl)phenyl-2(5H)-furanone reduced the increases in ARNA and PGE₂ by 43±5% and 47±8%, respectively. We conclude that cyclooxygenase-2 is expressed in the renal pelvic wall and that the activation of cyclooxygenase-2 contributes to the stimulation of renal mechanosensitive neurons in the pelvic wall. (Hypertension. 2000;35[part 2]:373-378.)

Key Words: prostaglandins ■ afferent renal nerves ■ cyclooxygenase ■ kidney ■ pelvis

Obstruction to urine flow (eg, ureteral stone, clot, or spasm) increases renal pelvic pressure. The increase in renal pelvic pressure distends the pelvic wall and activates mechanosensitive neurons in the pelvic wall, with a resultant increase in ipsilateral afferent renal nerve activity (ARNA).¹–⁵ The increase in ipsilateral ARNA decreases contralateral efferent renal nerve activity, which in turn increases contralateral urinary sodium excretion; this is known as the renal reflex response.¹

The kidney is one of the most active prostaglandin (PG)-producing tissues, with PGE₂ being one of the major metabolites.⁶ PGE₂ is a crucial mediator of the neural signal elicited by the increased renal pelvic pressure. Increasing renal pelvic pressure results in an increase in afferent renal nerve activity (ARNA) that is associated with increases in renal pelvic release of PGE₂ and substance P.³ Renal pelvic perfusion with the nonselective cyclooxygenase (COX) inhibitor indomethacin abolishes the increases in ARNA and renal pelvic release of PGE₂ and substance P produced through increased renal pelvic pressure.⁵ In vitro studies showed that PGE₂ increases the release of substance P from the renal pelvic wall via activation of N-type calcium channels.³ Further studies showed that the ARNA response to increased renal pelvic pressure is blocked by a substance P receptor antagonist.⁴ Taken together, these results suggest that stretching of the renal pelvic wall through increased renal pelvic pressure results in a release of PGE₂, which increases the release of substance P which in turn activates substance P receptors in the renal pelvic area with a resultant increase in ARNA.

COX is the principal enzyme in the synthesis of PG from arachidonic acid. Two isoforms of COX have been identified: a constitutive form, COX-1, and an inducible form, COX-2.⁷,⁸ COX-2 is undetectable in most tissues but can be induced by growth factors and various inflammatory agents. However, there is evidence for COX-2 being constitutively expressed in the central nervous system in areas involved in sensory processing.⁷ COX-2 has also been shown to be constitutively expressed in renal tissue.⁹–¹¹ Interestingly, changes in dietary sodium were found to alter the expression of COX-2 in the kidney.⁹,¹⁰,¹² suggesting an important role for COX-2 in the renal regulation of body water and sodium. A recent study on the urinary bladder showed that stretching of the bladder wall...
increased the expression of COX-2.13 Taken together, there is evidence to suggest that the COX isoformal involved in the activation of renal pelvic mechanosensitive neurons is COX-2. Therefore, we studied the expression of COX-2 in the renal pelvic wall by in situ hybridization histochemistry. Because these studies showed COX-2 expression in the renal pelvic wall, we examined the effects of 2 selective COX-2 inhibitors on the increases in ARNA and renal pelvic release of PGE2 produced through increased renal pelvic pressure.

Methods

The experimental protocols were approved by the Institutional Animal Care and Use Committee of the University of Iowa and performed according to the “Guide for the Care and Use of Laboratory Animals” from the National Institutes of Health.

The study was performed on male Sprague-Dawley rats weighing 190 to 388 g (mean weight 286±8 g). Anesthesia was induced with pentobarbital sodium (0.2 mmol/kg IP; Abbott Laboratories) and maintained with an infusion of pentobarbital sodium (0.04 mmol·kg⁻¹·h⁻¹ IV at 50 μL/min) into the femoral vein. Arterial pressure was recorded from a catheter in the femoral artery. The procedures for stimulating and recording ARNA have been previously described in detail.1,8 In brief, the left kidney was approached with a flank incision, a PE-10 catheter was placed in the right ureter for the collection of urine, and a PE-60 catheter was placed in the left ureter with its tip in the pelvis. A PE-10 catheter was placed inside the PE-60 catheter for the administration of vehicle and COX-2 inhibitors into the renal pelvis. Increasing renal pelvic pressure to stimulate ARNA was achieved by elevation of the PE-60 ureteral catheter above the level of the kidney. ARNA was recorded from the peripheral portion of the cut end of 1 renal nerve branch placed on a bipolar silver wire electrode. ARNA was integrated over 1-second intervals, with a unit of measure of microvolts per second per 1 second. Postmortem renal nerve activity, which was assessed through crushing of the decentralized renal nerve bundle peripheral to the recording electrode, was subtracted from all values of renal nerve activity. ARNA was expressed in percentage of its baseline value during the control period.1,8

Experimental Protocols

Approximately 90 minutes elapsed between the end of surgery and the start of the experiment to allow the rat to stabilize as demonstrated by 30 minutes of steady-state urine collections and ARNA recordings.

Effects of Increased Renal Pelvic Pressure on COX-2 mRNA Expression

Two groups of rats were studied. In the first group, renal pelvic pressure was increased by 30 mm Hg for 15 minutes. Two hours later, the kidneys were removed, immediately frozen in carbon dioxide, and stored at -80°C. The second group of rats, which served as a time control, were treated identically except renal pelvic pressure was not increased. Renal sections were cut at 14-μm thick with a cryostat (Microm), thaw-mounted onto ProbeOn slides (Fisher Scientific), and stored at -20°C until hybridization.

Labeling of the oligonucleotide probe for COX-2 mRNA and tissue hybridization was performed as previously described.14 Two oligonucleotide probes were mixed 1:1 before labeling. The oligonucleotide sequences were 5'-ATGGCATCAGTGTAGTAGTGGAGTGGGAGGCAC-3' (complementary to bases 1741 to 1791) and 5'-GATTAGTAC-TGTAG-GGTATTTGCTAATGTAGTGGTGGTGGAGGAC-3' (complementary to bases 1741 to 1791). The probes were labeled at the 3' end with 32P-dATP (New England Nuclear) with the use of terminal deoxynucleotidyltransferase (Amersham) in a buffer containing 10 mmol/L CoCl2, 1 mmol/L dithiothreitol (LKB), 300 mmol/L Tris base, and 1.4 mol/L potassium cacodylate (pH 7.2). The labeled probe was purified with the use of the QIAquick Nucleotide Removal Kit (Qiagen), and dithiothreitol was added to a final concentration of 10 mmol/L. The renal tissue sections were air dried and incubated for 16 to 20 hours at 42°C in a humidified box with hybridization solution containing the labeled probe. The hybridization solution contained 50% deionized formamide (J.T. Baker Chemicals BW), 4× SSC (1×=0.15 mol/L NaCl and 0.01 mol/L sodium citrate), Denhardt’s solution (0.02% BSA, 0.02% Ficoll [Pharmacia], and 0.02% polyvinylpyrrolidone), 0.2 mol/L NaPO4 (pH 7.0), 1% N-lauroylsarcosine, 10% dextran sulfate (Pharmacia), 500 μg/mL salmon testis DNA, and 200 mmol/L dithiothreitol (LKB). After hybridization, the sections were rinsed repeatedly with 1× SSC solution, transferred through distilled water, dehydrated in ethanol, and air dried. The slides were dipped in Kodak NTB2 liquid autoradiography emulsion, exposed in the dark at 4°C for 5 weeks, developed in Kodak D19, and fixed in Kodak 3000. Sections were rinsed in distilled water and coverslipped in glycerol. After examinations under dark-field illumination in a Nikon Microphot-FX microscope, the sections were counterstained with toluidine blue and coverslipped with Entellan (Merck) for examination under bright-field illumination. Photomicrographs were taken with Kodak T-max 100 film. Specificity of the probe was confirmed through hybridization of adjacent sections with a mixture of radiolabeled probe in the presence of a 100-fold excess of cold probe.

Effects of COX-2 Inhibition on the ARNA Response to Increased Renal Pelvic Pressure

The experiment consisted of 2 parts separated by a 10-minute interval. Each part consisted of a 20-minute control period, a 3-minute experimental period, and a 20-minute recovery period. Renal pelvic pressure was increased during each experimental period. Three groups were studied. In the first group (n=10), the renal pelvis was perfused with vehicle (0.1% DMSO) during the first part and the selective COX-2 inhibitor etodolac15 at 174 μmol/L during the second part. In the second group (n=10), the experimental protocol was identical except the renal pelvis was perfused with the selective COX-2 inhibitor 5,5-dimethyl-3-(3-fluorophenyl)-4-(4-methylsulfonyl) phenyl-2(5H)-furanone15 (DFU) at 28 μmol/L (n=5) or 140 μmol/L (n=5) instead of etodolac. The renal pelvic perfusates were switched immediately after the recovery period. Two different COX-2 inhibitors were administered to strengthen the hypothesis that the reduction in the PGE2 and ARNA responses to increased renal pelvic pressure produced by the 2 agents was related to inhibition of COX-2. The third group (n=6), serving as a time control, was exposed to the same experimental protocol, except the renal pelvis was perfused with vehicle throughout the experiment. In all 3 groups, a PE-50 catheter was inserted through the renal parenchyma into the renal pelvis to collect renal pelvic effluent for measurement of PGE2. The open end of the PE-60 ureteral catheter was clamped to allow drainage of all effluent via the PE-50 catheter inserted through the parenchyma. The pelvic effluent was collected on ice throughout the experiment and stored at -80°C for later analysis of PGE2.

Drugs

All agents were from Sigma, unless otherwise stated. DFU (Merck Frosst Canada, Inc, Center for Therapeutic Research) and etodolac were dissolved in DMSO and further diluted in 150 mmol/L NaCl to a final DMSO concentration of 0.1%.

Analytical Procedure

Sodium concentrations in the urine from the right contralateral kidney were determined with a flame photometer. Right urinary sodium excretion was expressed per gram kidney weight. Left urinary PGE2 concentration was determined by ELISA with a kit from Cayman Chemical Co.

Statistical Analysis

Systemic hemodynamics and renal excretion were measured and averaged over each period. The effects of increased renal pelvic pressure were calculated through a comparison of the experimental value with the average value of the bracketing control and recovery periods. Release of PGE2 into the renal pelvic effluent was calculated
as concentration multiplied by volume divided by duration of the collection period. A Wilcoxon matched-pairs signed rank test was used to test the significance between 2 related samples. A significance level of 5% was chosen. Values in the text and figures are expressed as mean±SEM.

Results

Effects of Increased Renal Pelvic Pressure on COX-2 mRNA Expression

An increase in renal pelvic pressure of 29±1 mm Hg for 15 minutes increased ARNA by 30±4% (P<0.05, n=5) in the experimental group. The results of in situ hybridization for COX-2 are shown in Figure 1. Prominent COX-2 mRNA signal was observed in the papilla and renal pelvic wall in both the time control group and the experimental group. The intensity of the COX-2 mRNA signal in the papilla and pelvic wall was greatest toward the lower portion of the pelvic cavity. The COX-2 mRNA signal was expressed in the interstitial cells in the papilla and in the subepithelial layer of the pelvic wall. Mean arterial pressure was similar in the control and experimental groups (104±3 and 107±4 mm Hg, respectively).

Effects of COX-2 Inhibition on the ARNA Response to Increased Renal Pelvic Pressure

PGE_2 plays an important role in the activation of renal mechanosensitive neurons. The presence of COX-2 mRNA in the renal pelvic wall (Figure 1), where the majority of the afferent renal neurons are located, suggested that activation of COX-2 contributes to the increased PGE_2 produced through increased renal pelvic pressure. We tested this idea by perfusing the renal pelvis with 2 different selective COX-2 inhibitors: etodolac and DFU. The results are shown in Figures 2 to 4. In the time control experiments, an increase in renal pelvic pressure by 21±0 mm Hg resulted in reproducible increases in ipsilateral ARNA, renal pelvic release of PGE_2 (Figure 2) and contralateral urinary sodium excretion from 1.4±0.4 to 2.4±0.7 and from 1.8±0.4 to 2.4±0.5 μmol·min⁻¹·g⁻¹, respectively (both P<0.05). In pilot experiments, we examined the effects of renal pelvic perfusion with etodolac at 35 μmol/L. Because these experiments showed that the ARNA response was not significantly reduced by etodolac at this concentration, with the ARNA response being 39±5% before and 34±10% (n=4) during etodolac, subsequent experiments were performed with etodolac at a 5-fold higher concentration (174 μmol/L). In the absence of etodolac, an increase in renal pelvic pressure by 21±0 mm Hg resulted in reversible increases in ARNA and renal pelvic release of PGE_2 (Figure 3) of similar magnitudes as in the time control experiments. Contralateral urinary sodium excretion increased from 1.3±2.0 to 2.0±0.6 μmol·min⁻¹·g⁻¹ (P<0.01). Renal pelvic perfusion with etodolac did not affect basal ARNA or basal renal pelvic release of

Figure 1. In situ hybridization of COX-2 from a kidney exposed to increased renal pelvic pressure. Bright-field exposure (A and C) shows papilla (PAP) with surrounding pelvic wall (PW). Dark-field exposure (B and D) shows strong COX-2 mRNA signal in interstitial cells in papilla and in subepithelial layer of pelvic wall 2 hours after exposure to a 15-minute period of increased renal pelvic pressure. Sections hybridized with excess cold probe showed no specific signal (not shown). Arrow depicts papilla in C. A and B ×120 magnification, C and D ×240 magnification.
PGE₂ but reduced the increases in ARNA (by 66±7%, P<0.01) and in renal pelvic release of PGE₂ (by 55±13%, P<0.01) produced through increased renal pelvic pressure. The increase in contralateral urinary sodium excretion was also blocked by etodolac (from 1.6±0.2 to 1.7±0.3 μmol·min⁻¹·g⁻¹, NS). Renal pelvic perfusion with DFU at 28 and 140 μmol/L resulted in similar effects, so the data were pooled. Before renal pelvic perfusion with DFU, an increase in renal pelvic pressure by 22±1 mm Hg resulted in similar increases in ARNA and renal pelvic release of PGE₂ (Figure 4) as in the other 2 groups. Renal pelvic perfusion with DFU did not affect basal ARNA or basal renal pelvic release of PGE₂ but reduced the increases in ARNA (by 43±5%, P<0.01) and in the renal pelvic release of PGE₂ (by 47±12%, P=0.02) produced through increased renal pelvic pressure. Mean arterial pressure (117±2, 110±2, and 107±2 mm Hg) and heart rate (314±8, 348±19, and 330±12 bpm) were similar in the 3 groups and were not altered with etodolac or DFU.

**Discussion**

The results of the in situ hybridization experiments show the presence of mRNA for COX-2 in the renal pelvic wall in anesthetized surgically operated rats. The functional studies show that the increases in ARNA and renal pelvic release of PGE₂ produced through increased renal pelvic pressure are blocked by renal pelvic perfusion with either etodolac or DFU, 2 selective COX-2 inhibitors. These data suggest that activation of COX-2 in the renal pelvic wall contributes to the stimulation of renal pelvic mechanosensitive neurons.

**COX-2 mRNA Expression in the Renal Pelvic Wall**

COX-2 is the inducible isofrom of COX in most tissues. However, there is evidence for COX-2 being constitutively expressed in the central nervous system and the kidney. The results of the current in situ hybridization of the kidney for COX-2 mRNA show a strong COX-2 mRNA signal in the...
papilla and renal pelvic wall in time control kidneys. Although the COX-2 mRNA signal observed in the time control kidney may reflect constitutively expressed COX-2, it cannot be excluded that COX-2, at least in part, was induced by the anesthesia and surgical procedures performed in the time control rats. Recent studies showing that dehydration induces marked upregulation of COX-2 expression in the inner medulla support this argument.11 Furthermore, only low levels of COX-2 mRNA were detected in the papilla and renal pelvic wall in kidneys harvested from rats immediately after the induction of anesthesia (K. Holmberg et al, unpublished observation). In agreement with previous studies,9 the present study shows that COX-2 was expressed in the interstitial cells in the inner medulla. In addition, there was marked expression of COX-2 mRNA in the smooth muscle layer of the renal pelvic wall, with the expression greatest in the lower part of the pelvis. We did not detect an increased COX-2 mRNA signal in the kidneys exposed to increased renal pelvic pressure compared with time control kidneys. It is possible that the duration of the increased renal pelvic pressure (15 minutes) was not sufficient to induce an increase in COX-2 mRNA. In the bladder, the maximum effect of urethral obstruction on COX-2 mRNA was observed after 6 hours of obstruction.13 On the other hand, it is conceivable that possible increases in COX-2 mRNA levels produced through increased renal pelvic pressure were obscured by the strong COX-2 mRNA signal present with basal renal pelvic pressure as shown in the time control kidneys. Nevertheless, the presence of a prominent COX-2 mRNA signal in the pelvic wall muscle layer, an area known to contain sensory neurons, suggests that the increased PGE2 synthesis produced through increased renal pelvic pressure was, at least in part, a consequence of activation of COX-2.

Role of COX-2 in the Activation of Renal Mechanosensory Neurons

There is considerable evidence that PGs enhance the responsiveness of sensory neurons to various stimuli, such as bradykinin.19 Likewise, in the kidney, PGE2 plays an important role in the activation of renal mechanosensitive neurons. The ARNA response to increased pelvic pressure is suppressed in arachidonic acid–deficient rats.5 Renal pelvic perfusion with indomethacin abolishes the increases in ARNA and renal pelvic release of PGE2 produced through increased renal pelvic pressure.2 PGE2 activates renal sensory neurons by increasing the release of substance P.2,3 In the kidney, the majority of the renal sensory nerve fibers are located in the pelvic wall smooth muscle layer, with the highest density toward the ureter and a diminishing number of fibers in the upper part of the pelvis.17,18 Our present findings show that the expression of COX-2 in the pelvic muscle wall was greatest in the lower portion of the pelvic cavity. These findings, together with those of our previous study that show that mechanical stimulation of an isolated renal pelvic wall preparation increases PGE2 release,2 suggest that COX-2 activation may contribute to the release of PGE2 and activation of sensory neurons through increased renal pelvic pressure. The results of the present study confirm this hypothesis. Renal pelvic perfusion with either etodolac or DFU,2 highly selective COX-2 inhibitors,15 reduced the increases in PGE2 release and ARNA produced with increased renal pelvic pressure. Our previous findings that indomethacin at a similar concentration (140 μmol/L) produced a more marked reduction in the increased release of PGE2 and ARNA elicited by increases in renal pelvic pressure suggest that activation of both COX-1 and COX-2 contributes to the activation of renal mechanosensitive neurons.

The inhibitory nature of the renorenal reflexes, as characterized by decreased efferent renal sympathetic nerve activity with increased urinary sodium excretion,1 suggests that the renorenal reflexes play a role in the renal control of body water and sodium balance. Renal medullary COX-2 expression is modulated by dietary sodium.10,11 A high sodium diet increases and a low sodium diet decreases medullary COX-2 expression. Although the effect of varying sodium diet on COX-2 expression in the renal pelvic wall has not been reported, our findings showing concurrent COX-2 mRNA
signals in both the inner medulla and the renal pelvic wall suggest that COX-2 expression in the pelvic wall is altered in parallel with the COX-2 expression in inner medulla. If so, we speculate that the renorenal reflexes may play a contributory role in promoting water and sodium excretion during high sodium diet. Increased COX-2 activity in the renal pelvic wall would enhance the responsiveness of renal mechanosensory neurons, ultimately leading to decreases in renal efferent sympathetic nerve activity and natriuresis.

In summary, the present data show the presence of COX-2 mRNA in the renal pelvic wall. Renal pelvic perfusion with 2 different selective COX-2 inhibitors reduced the increases in renal pelvic release of PGE$_2$ and ARNA. These data suggest that activation of COX-2 in the renal pelvic wall contributes to the stimulation of renal mechanosensitive neurons.

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