Selectivity of cyclooxygenase isoform activity and prostanoid production in normal and diseased Han:SPRD-cy rat kidneys

Lori Warford-Woolgar,1 Claudia Yu-Chen Peng,1 Jamie Shuhya,1 Andrew Wakefield,1 Deepa Sankaran,1 Malcolm Ogborn,1,2 and Harold M. Aukema1,2

Departments of 1Human Nutritional Sciences and 2Pediatrics and Child Health, University of Manitoba, Winnipeg, Manitoba

Submitted 17 August 2005; accepted in final form 12 October 2005

Warford-Woolgar, Lori, Claudia Yu-Chen Peng, Jamie Shuhya, Andrew Wakefield, Deepa Sankaran, Malcolm Ogborn, and Harold M. Aukema. Selectivity of cyclooxygenase isoform activity and prostanoid production in normal and diseased Han:SPRD-cy rat kidneys. Am J Physiol Renal Physiol 290: F897–F904, 2006.—Renal COX enzymes form prostaglandin H2 (PGH2) from arachidonic acid, which is released from renal membrane phospholipids by Ca2+–dependent cytosolic phospholipase A2 (cPLA2). PGH2 is then converted to PGE2, PGF2, and thromboxane A2 (TXA2) by synthases specific to each prostanoid. The specific roles of the two known COX isoforms in the kidney are not clear. They are known to be important in maintaining normal renal physiology, as well as playing a role in renal injury, as several studies demonstrated alterations in renal COX levels in kidney disease. In some models, such as experimental anti-Thy-1 glomerulonephritis, the expression of both COX-1 and COX-2 genes, as well as the cPLA2 gene, is upregulated as the disease progresses (21). In many models of renal disease, COX-2 inhibition appears to have beneficial effects (5, 11, 14, 16, 28, 38, 40, 57). COX-2 expression studies indicate that COX-2 metabolites are important in the regulation of hemodynamics, the renin-angiotensin system, and glomerulogenesis (18). Increased levels of COX-2 have been documented in adults with renal disease and in older experimental animal models of disease, and COX-2 inhibitors appear to be beneficial in these situations (45), while in other forms of renal injury they may not be beneficial (53). Other studies also have demonstrated a necessary role for COX-2 in kidney development (15, 33). Interestingly, in COX knockout mice, the expression of the intact COX isoform is not upregulated to compensate for the lack of the knocked out isoform, indicating that they have unique roles (32).

The kidney is a relatively rich source of prostanooids, which regulate renal processes such as hemodynamics, water and solute transport, renin secretion, and neurotransmitter release in the normal kidney. In the diseased kidney, prostanooids play a role in maintaining glomerular filtration rate (GFR) and salt/water homeostasis, as well as being involved in inflammatory and proliferative processes in response to renal injury. Due to the diversity of prostanooid effects, a reduction in prostanooid formation is associated with amelioration of the disease process in some types of renal disease, while in others it has a protective effect (8, 27). PGE2 and PGF2 have vasodilatory effects and play an important role in maintaining normal renal flow in the compromised kidney. Inhibition of prostanooid formation by nonsteroidal anti-inflammatory drugs has long been associated with nephrotoxicity (53). PGJ2 also increases potassium excretion via renin secretion, which ultimately leads to aldosterone secretion, while PGE2 is involved in the regulation of sodium reabsorption (18). PGF2 stimulates cell proliferation during repair, and inhibition of COX activity has been shown to impair this process and worsen renal injury (26). On the other hand, chronic progressive renal injury is often associated with overproliferation of renal cells, formation of fibrous tissue, and inflammation (37). Furthermore, because PGE2 is also involved in inflammatory responses and TXA2 is a potent vasoconstrictor, inhibition of prostanooid formation can reduce renal injury (5, 11, 14, 16, 38, 40, 51, 57). Hence, the

Adolesceurogenase (COX)-1 and COX-2 are expressed in the rat and human kidney. Renal COX-1 in the rat is found in glomerular mesangial cells, cortical and medullary collecting tubules, and the renal vasculature, whereas COX-2 is present in the macula densa and surrounding cortical thick ascending limb and in medullary interstitial cells (10, 19, 30, 48, 49). COX enzymes form prostaglandin H2 (PGH2) from arachidonic acid, which is released from renal membrane phospholipids by Ca2+–dependent cytosolic phospholipase A2 (cPLA2). PGH2 is then converted to PGE2, PGF2, and thromboxane A2 (TXA2) by synthases specific to each prostanoid.

The specific roles of the two known COX isoforms in the kidney are not clear. They are known to be important in

1 Address for reprint requests and other correspondence: H. M. Aukema, HS06 Duff Roblin Bldg., Dept. of Human Nutritional Sciences, Univ. of Manitoba, Winnipeg, Manitoba, Canada, R3T 2N2 (e-mail: aukema@umanitoba.ca).

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effects of prostanoids are often conflicting and the relative balance between them determines overall effects.

The relative contribution of COX isoform activity to renal prostanoid production or whether these prostanoids are produced in an isoform-specific manner is not known. With the widespread use of COX isoform-specific inhibitors, knowing which renal prostanoids are produced by specific isoforms is necessary to understand the potential effects of these inhibitors on the kidney, particularly when kidney function is already compromised by disease. In the Han:SPRD-cy rat model of renal cyst disease, we showed that the protein expression of the COX isoforms is altered (2). The objectives of the current studies, therefore, were to determine 1) the relative contribution of the COX isoforms on renal prostanoid production in the normal kidney, 2) whether the production of prostanoids is specific to each COX isoform, and 3) whether a genetically determined form of renal disease alters COX isoform protein expression, activity, and the prostanoid profile.

MATERIALS AND METHODS

Animal model. Normal rats (+/+) and littersmates with chronic kidney disease (Cy/+ ) were obtained from our colony of Han:SPRD-cy rats, which was derived from breeding pairs that were kindly provided to us by Dr. B. Cowley, University of Oklahoma Health Sciences Center, Oklahoma City, OK (12). This model of renal disease was established as a model of polycystic kidney disease as the diseased rats develop many renal cysts. By 8 wk of age, significant cyst formation can be observed, preferentially located in proximal tubules of the juxtamedullary nephrons of the inner cortex with less being observed in the outer cortex and medulla (12, 35, 36, 41). Moderate increases in blood pressure also have been observed in these rats in some studies, but not in others (1, 12, 25, 41). Disease progression ultimately results in tubulointerstitial injury, interstitial fibrosis, and inflammation, pathological features that eventually are found in all forms of chronic renal disease. For establishment of conditions of COX activity assays, 3- to 6-mo-old breeders were used.

For determination of effects of chronic renal injury on prostanoid production and COX activities, 10- to 12-wk-old normal and diseased littersmates were used. At termination, rats were weighed before being asphyxiated with an overdose of CO2 and collection of blood and tissues. All procedures were approved by the University of Manitoba internal review board and followed the guidelines of the Canadian Council on Animal Care. Serum urea and serum and urine creatinine were measured using commercial kits (Sigma, Oakville, Canada) and contains the COX proteins. Protein concentrations of all fractions were determined using the Bradford method (6). After SDS-PAGE, proteins were transferred to nitrocellulose paper and blocked with 5% non-fat milk in TBS-T buffer for 1 h and incubated with primary antibodies (Cayman Chemical) overnight at 4°C with primary antibodies (Cayman Chemical) diluted to 1:20,000 to 1:250, followed by incubation for 1 h at room temperature with a peroxidase-conjugated secondary antibody at a dilution of 1:20,000 (2). Immunoblots were incubated with ChemiGlow (Alpha Innotech, Lake Forest, CA) and scanned and analyzed using ImageQuant software (Molecular Dynamics, Sunnyvale, CA). The relative abundance of COX-1 and COX-2 protein expression was determined by densitometry.

Prostanoid production and COX activity. Sixty milligrams of lyophilized tissue from the right kidney were homogenized in 2 ml of fresh Tyrode buffer on ice. Triton X-100 was then added to achieve a final concentration of 1% ethanol as vehicle control or carrier for SC-560. The times chosen were based on time course incubations (Fig. 1) and the level of inhibitor used to inhibit COX-1 was based on published IC50 values and confirmed for the rat using a whole blood assay. At the end of the incubation period, reactions were stopped with 800 μl of ice-cold acetylsalicylic acid (5 mmol/l final concentration). Samples were then vortexed and centrifuged at 12,000 g at 4°C for 5 min. The supernatant was collected and stored at −80°C for the determination of PGE2 and the stable metabolites of PGF2α (6-keto-PGF1α) and TxA2 (TXB2) using commercial enzyme immunoassay kits (Cayman Chemical).

Whole blood assay. COX isoform inhibition by SC-560 or niflumic acid in the rat was determined by measuring TXB2 production in rat whole blood as described (9, 43). Briefly, blood was collected via heart puncture from anesthetized (90/10 mg/kg ip ketamine/xylazine) adult male rats into heparanized tubes. Aliquots of 500 μl were added to siliconized microcentrifuge tubes containing final concentrations of the following: SC-560 varying from 0.0001 to 100 μM or niflumic acid varying from 0.01 to 170 μM, and 20 μg/ml calcium ionophore. All samples were incubated for 15 min at 37°C before calcium ionophore was added, and for another 60 min after the calcium ionophore was added. At the end of the incubation period, samples were centrifuged for 15 min at 1,000 g at 4°C. The supernatant was removed, and 100 μl were mixed with 400 μl of HPLC grade methanol to precipitate proteins. The samples were then centrifuged for 5 min at 12,000 g at 4°C, and supernatants were collected and stored at −80°C for TXB2 determination using the enzyme immunoassay kit as described above.

Immunoblotting. Half of the left kidney was lyophilized and 30 mg were homogenized in 100 Vol of ice-cold homogenization buffer containing protease inhibitors as described (2). Briefly, homogenates were centrifuged at 100,000 g for 30 min at 4°C and the supernatant, which represents the cytosolic fraction, was removed. The remaining pellet was resuspended in 15 Vol of homogenization buffer containing 1% Triton X-100 (Sigma, St. Louis, MO), incubated on ice for 10 min, and centrifuged at 100,000 g for 30 min at 4°C. The resulting supernatant was collected as the particulate fraction, which represents the Triton-soluble fractions of the plasma and intracellular membranes and contains the COX proteins. Protein concentrations of all fractions were determined using the Bradford method (6). After SDS-PAGE, detection of COX-1 and COX-2 was carried out by incubating blots overnight at 4°C with primary antibodies (Cayman Chemical) diluted to 1:250, followed by incubation for 1 h at room temperature with a peroxidase-conjugated secondary antibody at a dilution of 1:20,000 (2). Immunoblots were incubated with ChemiGlow (Alpha Innotech, Lake Forest, CA) and scanned and analyzed using ImageQuant software (Molecular Dynamics, Sunnyvale, CA). The relative abundance of COX-1 and COX-2 protein expression was determined by densitometry.

Fig. 1. Time course curves for the in vitro renal production of thromboxane B2 (TXB2) (●), PGE2 (■), and 6-keto-PGF1α (▲). Kidneys from normal adult rats were lyophilized, homogenized in Tyrodes buffer, and incubated at 37°C for 0–80 min (n = 4).
San Leandro, CA) and image analysis and quantitation of immunoreactive bands were performed using the FluorChem FC digital imaging system (Alpha Innotech). A reference kidney homogenate was loaded on each gel in duplicate so that results could be compared across gels. Dose-response curves were used to determine the linear range of response (16–20 mg of protein was used).

**Real-time RT-PCR.** Total RNA was extracted from 10–20 mg of lyophilized kidneys using TRIzol. DNA was removed by DNase treatment with DNase I (Invitrogen, Carlsbad, CA) for 15 min at room temperature. One-step RT-PCR was performed on 0.5 μg of total RNA using the Quantitect SYBR Green RT-PCR kit (Qiagen, Mississauga, Canada). PCR primers were chosen using Primer 3 software (39). Oligonucleotide sequences were as follows: COX-1 sense, 5'-GCTTGGCACCTGTTGGT-3', COX-1 antisense, 5'-AGGTTGGATTCTTACAAACTCC-3', COX-2 sense, 5'-TACCCGGACTG-GATTTCTACG-3', and COX-2 antisense, 5'-TTGGAAGGAGGAATGTTG-3'. Real-time RT-PCR was performed with a Cepheid SmartCycler II (Cepheid, Sunnyvale, CA) with the following protocol: reverse transcription at 50°C for 30 min, PCR activation at 95°C for 15 min, 40 PCR cycles at 94°C for 15 s, 55°C for 30 s, and 72°C for 30 s. Relative amounts of mRNA were determined by comparing cycle threshold (CT) numbers for equal amounts of RNA subjected to PCR and calculating the difference between normal and diseased kidney mRNA expression from the CT difference using the formula 2^{ΔCT}.

**Statistical analysis.** To determine differences between data from normal and diseased kidneys, data were tested by Student’s t-test if variances were not equal, as determined by the t-test if variances were not equal, as determined by the Folded F method (SAS, Cary, NC).

**RESULTS**

**Normal rat kidney prostanoids and COX isoforms.** Time course studies of prostanoid production showed that PGE₂, 6-keto-PGF₁α, and TXB₂ were present in the kidney at time 0, with production increasing rapidly and reaching steady-state levels by 30 to 40 min in this in vitro assay (Fig. 1). From these time course studies, a 10-min incubation period was used for the COX activity assays and 60 min was used to determine steady-state levels of prostanoids in this assay system.

To determine whether prostanoid production is specific to COX isoform activity in the kidney, SC-560, a selective COX-1 inhibitor, was used. The whole blood assay confirmed that the SC-560 IC₅₀ of 0.009 μM for human COX-1 was similar for rat COX-1 (Fig. 2A) and that at a concentration of 0.1 μM, more than 90% of rat COX-1 activity is inhibited. This concentration is ×5 lower than the reported IC₅₀ for COX-2. When kidney homogenates were incubated with SC-560, COX activity was inhibited between 1 and 10 μM, corresponding to the IC₅₀ of 6.3 μM for COX-2 (Fig. 2B). There was no inhibition of COX activity at the IC₅₀ for COX-1 (0.009 μM), demonstrating that the predominant COX activity in the kidney is due to the COX-2 isoform. This was confirmed using the selective COX-2 inhibitor niflumic acid (Cayman Chemical), which has a COX-2 IC₅₀ of 0.1 μM and a COX-1 IC₅₀ of 16 μM. TXB₂ production in kidney homogenates is almost completely inhibited at 4 μM niflumic acid, a concentration greater than the COX-2 IC₅₀, but below the COX-1 IC₅₀ (Fig. 3, A and B). Because the selectivity is greater for SC-560 compared with niflumic acid, SC-560 was chosen as the selective inhibitor for the remaining studies reported herein.

**Prostanoids and COX isoforms in disease.** To determine the effect of renal disease on prostanoid production and COX isoform protein levels and activity, kidneys from normal and diseased Han:SPRD-cy rats were analyzed. Consistent with previous reports (1, 2, 12, 25, 35, 36, 41), kidneys from diseased rats were more than three times larger than normal kidneys, reflecting the presence of renal cysts. Deterioration of renal function is reflected in the higher serum urea and creatinine levels, increased proteinuria, and lower creatinine clearance in diseased compared with normal rats (Table 1). Immunoblotting analyses show that COX-1 protein levels were elevated by 117% (P < 0.0001), whereas COX-2 protein levels were 93% lower (P < 0.0001) in diseased kidneys compared with normals (Fig. 4). These patterns were reflected in the mRNA levels, although only the lower expression of COX-2 mRNA in diseased kidneys was statistically significant (Fig. 5).

In normal kidneys, PGE₂ and 6-keto-PGF₁α were the predominant renal prostanoids, accounting for more than 90% of the endogenous and steady-state in vitro levels following a 60-min incubation (Table 2). The prostanoid profile was similar in normal kidneys for endogenous and steady-state in vitro levels (50 and 69% 6-keto-PGF₁α, 42 and 27% PGE₂, and 8 and 5% TXB₂, respectively, of total prostanoids). These levels were all higher in diseased kidneys, with the differences between normal and diseased kidneys ranging from less than 2 times (endogenous PGE₂ levels) to almost 10 times (steady-state in vitro TXB₂ levels). The differences were greatest for
COX ISOFORMS AND PROSTANOIDS IN NORMAL AND DISEASED KIDNEYS

Fig. 3. TXB₂ production in the presence of the COX-2-selective inhibitor niflumic acid in calcium ionophore-stimulated whole blood (A) and kidney homogenates (10-min incubations) from normal adult rats (n = 2; B).

TXB₂ and lowest for PGE₂, resulting in a shift in the prostanoid profile in diseased kidneys for the endogenous and steady-state in vitro levels (60 and 74% 6-keto-PGF₁α, 27 and 14% PGE₂, and 13 and 11% TXB₂, respectively, of total prostanoids).

The prostanoid profile resulting from COX activities was similar to the prostanoid levels (Table 3). COX activities resulting in 6-keto-PGF₁α, PGE₂, and TXB₂ production represented ~60–66, 30, and 8% of total prostanoids, respectively, in normal kidneys and ~72, 11–14, and 16% in diseased kidneys. As with the prostanoid levels, the differences in COX activities between normal and diseased kidneys ranged from not significantly different (COX-1 determined by PGE₂ production) to more than eightfold higher (COX activity determined by TXB₂ production). Both COX-1 and COX-2 activities were higher in diseased kidneys when determined by TXB₂ and 6-keto-PGF₁α production. However, when determined by PGE₂ production, COX-2 activity was significantly higher in diseased compared with normal kidneys, whereas COX-1 activity was not different.

To examine whether the relative COX isoform activities were different in diseased compared with normal kidneys, the ratios of COX-1/COX-2 activities were determined (Fig. 6). These ratios were higher when these activities were determined by TXB₂ or 6-keto-PGF₁α production, but not for PGE₂ production. Thus, although the predominant COX activity was still due to the COX-2 isoform, the relative change was greater for COX-1 than COX-2, when TXB₂ or 6-keto-PGF₁α was used to measure the respective COX activities.

To determine which prostanoid exhibited the greatest difference between diseased compared with normal kidneys, ratios of the prostanoids were calculated and compared (Fig. 7). TXB₂/PGE₂ was higher in diseased compared with normal kidneys when calculated by endogenous or steady-state in vitro levels, by prostanoids resulting from both COX isoform activities or from total COX activity. Similarly, TXB₂/6-keto-PGF₁α also was higher in diseased compared with normal kidneys for all conditions except when production of these prostanoids resulted from COX-1 activity. Hence, the difference in levels and production of prostanoids in diseased compared with normal kidneys was greater for TXB₂ than for either 6-keto-PGF₁α or PGE₂. The ratio of 6-keto-PGF₁α/PGE₂ also was higher in diseased compared with normal kidneys for all conditions. Therefore, the differences in the increased levels of prostanoids in diseased compared with normal kidneys were in the order of TXB₂ > 6-keto-PGF₁α > PGE₂.

DISCUSSION

This study demonstrates that in the normal rat kidney the COX-2 isoform is responsible for most of the potential COX activity, with very little detectable COX-1 activity. Further-

Table 1. Animal and kidney weights and serum chemistry in normal (+/+) and diseased (Cy/+ rat kidneys

<table>
<thead>
<tr>
<th></th>
<th>(+/+)</th>
<th>Cy/+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt, g</td>
<td>356±5</td>
<td>339±5*</td>
</tr>
<tr>
<td>Kidney wt, g</td>
<td>2.64±0.05</td>
<td>3.33±0.33†</td>
</tr>
<tr>
<td>Kidney wt, g/100 g body wt</td>
<td>0.74±0.01</td>
<td>2.46±0.09‡</td>
</tr>
<tr>
<td>Serum urea, μmol/l</td>
<td>7.7±1.2</td>
<td>30.8±1.9‡</td>
</tr>
<tr>
<td>Serum creatinine, μmol/l</td>
<td>60±12</td>
<td>114±13†</td>
</tr>
<tr>
<td>Creatinine clearance, μl·min⁻¹·100 g body wt⁻¹</td>
<td>304±39</td>
<td>144±20‡</td>
</tr>
<tr>
<td>Urine protein, mg/24 h</td>
<td>9.1±0.7</td>
<td>12.6±1.1†</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05, †P < 0.01, ‡P < 0.001, significantly different from normal. §n = 8–9 for urine protein.
more, in diseased compared with normal kidneys of Han:SPRD-cy rats, the higher COX activity is due primarily, although not exclusively, to COX-2.

In apparent contrast to the activity levels of these COX isoforms, the localization of immunoreactive protein and mRNA expression for COX-1 in the kidney is more widespread than for COX-2. COX-1 is constitutively expressed in the collecting ducts, the mesangium, and the vasculature of the rat kidney, whereas COX-2 expression is limited to the macula densa, the loop of Henle adjacent to the macula densa, and medullary interstitial cells (10, 19, 30, 48, 49). Because of the wider distribution of COX-1-immunoreactive protein and mRNA expression, and because COX-1 is regarded as the major and constitutive isoform while COX-2 is predominantly present as the inducible form in most other tissues, it is has been concluded that COX-1 is the main source of prostanoids in the kidney (13, 43). The activity levels of these isoforms in the current study, however, suggest that COX-2 activity produces the majority of the prostanoids in rat kidney.

Studies of selective COX inhibitor effects in the kidney support the importance of COX-2 activity compared with COX-1 activity in the kidney. Renal PGE$_2$ production is increased by renal ablation and reduced by COX-2-selective but not COX-1-selective inhibitors (51). In another study, the selective COX-2 inhibitor NS-398 reduced GFR in streptozotocin-induced rats while the selective COX-1 inhibitor valeryl salicylate had no effect on this parameter (28). Interestingly, both inhibitors attenuated the increase in urinary PGE$_2$ observed in streptozotocin-induced rats not treated with inhibitor, but only NS-398 attenuated the excretion of TXB$_2$, thus implying that in this animal model of renal disease COX-2 plays a major role in TXB$_2$ production. In agreement with this, in the current study COX-2 activity produces most ($80\%$) of the higher amounts of TXB$_2$ in diseased rat kidneys, with a smaller contribution from COX-1 activity. PGE$_2$ production in medullary interstitial cells is inhibited by a COX-2-selective but not a COX-1-selective inhibitor, consistent with the localization of the COX-2 isoform to these cells (17). A number of other studies in various renal models, although not determining COX activities, demonstrate altered renal function and reduced disease progression when COX-2 inhibitors are administered, further supporting the importance of this COX isoform in renal health and disease (11, 14, 16, 38, 40, 51, 57). The fact that targeted deletion of COX-2, but not COX-1, results in abnormal renal development also gives credence to the importance of COX-2 in the kidney (15, 33). Furthermore, human studies with COX-2-selective inhibitors indicate that inhibition of COX-2 activity in the kidney results in adverse renal effects such as reduced GFR and sodium excretion (7, 23, 44).

In the majority of reports, the presence of renal disease increases the expression of COX protein and mRNA and this parallels increased enzyme activity (21, 22, 28, 29, 50, 51, 52).

### Table 2. Endogenous and steady-state in vitro levels (ng/mg protein) of TXB$_2$, PGE$_2$, 6-keto-PGF$_{1α}$, and total prostanoids in normal (+/+) and diseased (Cy/+ ) rat kidneys

<table>
<thead>
<tr>
<th></th>
<th>Endogenous Levels (n = 14–18)</th>
<th>Steady-State In Vitro Levels (n = 14–18)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>+/+</td>
<td>Cy/+</td>
</tr>
<tr>
<td>TXB$_2$</td>
<td>0.30±0.05</td>
<td>1.32±0.08†</td>
</tr>
<tr>
<td>PGE$_2$</td>
<td>1.57±0.40</td>
<td>2.71±0.29*</td>
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<tr>
<td>6-keto-PGF$_{1α}$</td>
<td>1.90±0.29</td>
<td>5.97±0.53†</td>
</tr>
<tr>
<td>Total</td>
<td>3.77±0.65</td>
<td>10.00±0.78†</td>
</tr>
<tr>
<td></td>
<td>+/+</td>
<td>Cy/+</td>
</tr>
<tr>
<td>TXB$_2$</td>
<td>0.65±0.09</td>
<td>6.43±0.64†</td>
</tr>
<tr>
<td>PGE$_2$</td>
<td>3.44±0.42</td>
<td>8.01±0.99†</td>
</tr>
<tr>
<td>6-keto-PGF$_{1α}$</td>
<td>8.74±0.72</td>
<td>41.79±3.07†</td>
</tr>
<tr>
<td>Total</td>
<td>12.72±1.13</td>
<td>56.45±4.05†</td>
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Values are means ± SE. *P < 0.01, †P < 0.001, significantly different from normal.
In the current study, there was consistency between levels and activity for COX-1; however, this was not the case for COX-2. COX-2 protein and gene expression were markedly reduced in diseased compared with normal kidneys but the activity of COX-2 ranged from one to seven times higher, depending on which prostanoid was used to measure enzyme activity. A similar finding has been reported in 5/6 nephrectomized rats in which celecoxib administration resulted in a tripling of immunoreactive COX-2 in the macula densa while COX-2 activity was reduced in vivo, as evidenced by reduced urinary prostanoid excretion. Although immunohistochemistry is not ideal for quantitation of protein expression, renal COX-2 protein levels were found to be three times higher in rats administered the COX-2 inhibitor (16). A reduction in enzyme level with an increase in activity suggests that feedback inhibition by prostanoids may be one of the regulatory mechanisms controlling the protein levels of COX-2, at least in the macula densa. However, this issue is not clear, as many studies have demonstrated parallel COX-2 expression and enzymatic activity in the partially nephrectomized rat and other models of renal injury (21, 22, 28, 29, 50, 51). It is possible that the depletion of normal macula densa cells in the Han:SPRD-cy rat, as reported by Al-Nimri et al. (1), results in less COX-2 expression and that other parts of the kidney contribute to the higher overall COX-2 activity, in an analogous fashion to the study by Fujihara et al. (16). Han:SPRD-cy rats exhibit focal cyst development in the cortex, extending to the medulla only occasionally (12, 35, 36, 41); therefore, the observed increased renal COX-2 activity in diseased rats may originate from the relatively preserved medulla. Indeed, changes in COX-2 levels in response to altered ion concentration depend on its localization within the kidney. Hypertonic states increase COX-2 mRNA expression and immunolabeling in the inner medulla.

### Table 3. Renal COX, COX-1, and COX-2 activities as determined by PGE2, 6-keto-PGF1α, TXB2, and total prostanoid production in normal (+/+ and diseased (Cy+/+) rat kidneys

<table>
<thead>
<tr>
<th></th>
<th>COX Activity (n = 14–18)</th>
<th>COX-1 Activity (n = 14–18)</th>
<th>COX-2 Activity (n = 14–18)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Cy/+</td>
<td>Cy/+</td>
<td>Cy/+</td>
</tr>
<tr>
<td>TXB2</td>
<td>0.05±0.01</td>
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<tr>
<td>PGE2</td>
<td>0.18±0.03</td>
<td>0.02±0.01</td>
<td>0.20±0.05</td>
</tr>
<tr>
<td>6-keto-PGF1α</td>
<td>0.41±0.05</td>
<td>0.03±0.01</td>
<td>0.39±0.05</td>
</tr>
<tr>
<td>Total</td>
<td>0.62±0.08</td>
<td>0.05±0.02</td>
<td>0.64±0.10</td>
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</table>

Data are means ± SE expressed as ng·mg protein⁻¹·min⁻¹. *P < 0.01, †P < 0.001, significantly different from normal. COX, cyclooxygenase.
and decrease COX-2 mRNA expression and immunolabeling in the cortex (24, 31, 46, 55, 56). Further complicating this issue, increased COX-2 activity in diseased kidneys could be due to increased macrophage infiltration in these kidneys (35, 36), as has been observed in lupus nephritis (45). Analyses of COX isoform activities in distinct microdissected segments of the nephrons or immunohistochemical localization of normal and diseased kidneys from Han:SPRD-cy rats are required to elucidate the specific roles of each COX isoform in specific parts of the nephron. These types of studies may help explain the apparent discrepancy between total renal COX-2 enzyme expression and activity in this model.

Although the predominant activity in diseased kidneys is due to the COX-2 isoform, compared with normal kidneys, there is increased COX-1 activity and the relative difference in COX isoform activity is greater for COX-1 than COX-2, as evidenced by the higher COX-1/COX-2 ratios in diseased kidneys compared with normals. Hence, while COX-1 activity appears to play a minimal role in the normal kidney, its importance appears to be greater in the diseased kidney. Although in most animal models of renal disease it is the COX-2 isoform that is elevated in disease (22, 28, 29, 40, 52), there are some instances where COX-1 is increased (4, 20), and inflammatory cytokines have been demonstrated to increase COX-1 in rat glomerular mesangial cells (47).

In addition to the selective changes in COX isoform activities, selective changes in prostanoid production also are observed. Compared with normal kidneys, the ratios of TXB2/6-keto-PGF1α, TXB2/PGE2, and 6-keto-PGF1α/PGE2 are higher in diseased kidneys, indicating that TXB2 is altered more by disease than either 6-keto-PGF1α or PGE2 and that among the latter 6-keto-PGF1α is altered more than PGE2 by disease. Hence, because the effect of disease on prostanoid production varies depending on which prostanoid is being examined, it is important to specify which prostanoid is being analyzed when determining effects of prostanoid-altering interventions in the kidney. This is further illustrated in the determinations of COX activities in normal and diseased kidneys. Depending on which prostanoid is being used to determine COX activity, or which isoform activity is being determined, the difference in COX activity between normal and diseased kidneys ranges from not significantly different to more than 10-fold different.

The current data are not suggestive of different prostanoid production by the COX isoforms. TXB2, PGE2, and 6-keto-PGF1α production makes up 16, 11, and 73%, respectively, of the COX-1 activity in diseased kidneys. This is similar to the 16, 14, and 71% contribution of TXB2, PGE2, and 6-keto-PGF1α to the COX-2 activity in these kidneys. The similar prostanoid profiles resulting from the COX isoforms in contrast to differences in the prostanoid profiles in normal compared with diseased kidneys suggest that the effect of disease on prostanoid production may be due to alterations in prostanoid-specific synthase enzymes. Future studies to explore the colocalization of COX isoforms and specific prostaglandin synthases would aid in elucidating this possibility.

The specific localization of COX isoforms in the kidneys suggests that the selective changes in COX isoforms and prostaglandin production in rat renal disease as described herein would have specific effects on the kidney (10, 19, 30, 48, 49). The higher levels of COX-1 activity would result in higher levels of prostanooids in the renal vascular tissue, mesangium, and/or collecting tubules, while increased COX-2 would increase prostanoid levels in the macula densa, the surrounding loop of Henle and/or medullary interstitial cells. TXA2 is a vasoconstrictor and associated with reduced renal blood flow and kidney damage (3, 34, 54), whereas the prostaglandins are vasodilators (42), so the balance of these prostanooids would dictate the net physiological effect. In diseased kidneys, the TXB2 to prostaglandin ratios are higher than in normals, suggesting that the balance shifts toward a greater vasoconstrictory effect in diseased kidneys.

In conclusion, COX-2 activity is the major form of COX activity in the normal rat kidney. Although some of the higher COX activity in diseased Han:SPRD-cy rat kidneys is attributable to COX-1, the majority of the total COX activity is due to the COX-2 isoform in disease as well. The increased COX activity results in a shift in the balance of prostanooids to a more vasoconstrictory state and may contribute to the increased disease progression. As selective COX inhibitors affect other forms of renal disease, selective inhibition of COX activity in the Han:SPRD-cy rat appears worthy of examination. Because 10% of patients with end-stage renal disease have renal cyst disease, benefits observed in this model may be relevant to individuals afflicted with this disorder.

ACKNOWLEDGMENTS

The authors thank L. Evans for technical assistance with immunoblotting analyses.

GRANTS

This research was supported in part by grants from the Natural Sciences and Engineering Research Council of Canada, the Dairy Farmers of Canada, and a University of Manitoba Graduate Fellowship (to L. Warford-Woolgar).

Present address of L. Warford-Woolgar: Dept. of Biochemistry, Memorial Univ. of Newfoundland, St. John’s, NL, Canada A1B 3X9.

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