Intrarenal expression and distribution of cyclooxygenase isoforms in rats with experimental heart failure

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Received 30 August 1999; accepted in final form 5 September 2000

Abassi, Zaid, Sergey Brodsky, Olga Gealekman, Irith Rubinstein, Aaron Hoffman, and Joseph Winaver. Intrarenal expression and distribution of cyclooxygenase isoforms in rats with experimental heart failure. Am J Physiol Renal Physiol 280: F43–F53, 2001.—The generation of PGs from arachidonic acid is mediated by cyclooxygenase (COX), which consists of a constitutive (COX-1) and an inducible (COX-2) isoform. The present study evaluated the relative expression and immunoreactive levels of COX-1 and COX-2, by means of RT-PCR, Western blot analysis, and immunohistochemistry, in the renal cortex and medulla of rats with congestive heart failure (CHF), induced by the placement of an aortocaval fistula. In addition, we examined the effects of a COX-1 inhibitor (piroxicam), COX-2 inhibitor (nimesulide), and nonselective COX inhibitor (indomethacin) at a dose of 5 mg/kg, on intrarenal blood flow by laser Doppler flowmetry. COX-1 and COX-2 mRNAs were abundantly expressed in the renal medulla of control and CHF rats and only minimally in the cortex. Moreover, both RT-PCR (32–36 cycles) and Western blot techniques revealed upregulation of medullary COX-2, but not of COX-1, in rats with advanced heart failure. In line with these findings, all three tested COX inhibitors provoked significant and sustained decreases (Δ ~ −20%) in medullary blood flow (MBF), which were similar in magnitude and duration in control animals. However, in CHF rats, indomethacin produced a greater reduction in MBF than that obtained with either piroxicam or nimesulide. Taken together, these results indicate that 1) both COX-1 and COX-2 are predominantly expressed in the renal medulla and 2) experimental CHF is associated with selective overexpression of COX-2. The latter may represent a mechanism aimed at defending MBF in the face of a decrease in renal perfusion pressure during the development of CHF.

congestive heart failure; cyclooxygenase; renal hemodynamics; rat

Cyclooxygenase (COX), also known as prostaglandin H-endoperoxide synthase, is a key enzyme in the synthesis of PGs from arachidonic acid (AA). It has recently become apparent that there are two different isoforms of COX, referred to as COX-1 and COX-2, that are encoded by different genes (29). The two isoforms share similar enzymatic properties and exhibit similar Michaelis-Menten constant (Km) values for their substrate, AA. However, they differ markedly with respect to cellular expression and regulation. COX-1 is expressed constitutively in a wide variety of tissues and is thought to be responsible for the continuous generation of PGs. In contrast, the expression of COX-2 is restricted to certain cell types but can be dramatically increased during inflammation and by a variety of cytokines or growth factors (29, 34). Recent studies have demonstrated that both isoforms of COX are present in the kidney (11, 13, 18, 36). COX-1 is constitutively expressed in the glomerulus, the thick ascending limb of Henle’s loop, and all portions of the collecting duct (36). Interestingly, in contrast to many other tissues, COX-2 is also constitutively expressed in the kidney, in particular in the macula densa cells (13), the renal medulla (13, 31, 36), and the thick ascending limb (30). Guan et al. (11) demonstrated in rabbit cultured medullary interstitial cells that COX-2 mRNA predominated over that of COX-1, suggesting that the renal medulla is a major site of COX-2-mediated PGE2 generation in vivo. Similar results were reported by Ferguson et al. (10), who demonstrated that COX-2 is the major COX isoform contributing to PGE2 synthesis by a medullary collecting duct cell line. However, the relative contributions of the two isoforms to overall renal production of the autacoids are largely unknown at present. Indeed, recent studies have shown that the renal expression of COX-2, rather than of COX-1, can be modulated by changes in dietary sodium and sodium balance, as well as by changes in medullary tonicity (13, 35, 36).

PGs play an important role in the regulation of renal hemodynamics and in the transport of salt and water along the nephron (20, 21). PGE2, the major metabolic product of this group in the rat kidney, exerts a vasorelaxant effect on glomerular afferent arteriole and mesangial cells, thereby potently modulating renal blood flow (RBF) and glomerular filtration rate (GFR). In the
renal medulla, where PGs originate primarily from two 
main cell types, the renal medullary interstitial cells 
and the epithelial cells of the collecting duct, both 
isoforms of COX are abundantly expressed (13, 36).
The local production of PGE_2 in the renal medulla is 
considered to be of importance in the preservation of 
adequate blood flow to the medulla, a region on the 
 verge of tissue hypoxia under normal conditions, due 
to a limited blood supply (3). Indeed, numerous studies 
have demonstrated that COX inhibition is associated 
with a substantial decrease in medullary blood flow 
(MBF) (2, 19, 26).

The renal vasodilatory effects of PGE_2 and perhaps 
prostacyclin (PGI_2) are of particular importance in 
pathophysiological situations associated with a de-
crease in effective circulating volume, which are char-
acterized by activation of neuro-humoral vasoconstric-
tor systems (37). In these situations, PGE_2 is thought 
 to play a crucial role in the protection of RBF and GFR 
by virtue of its ability to antagonize the renal vasocon-
strictor action of ANG II and norepinephrine, thus 
maintaining adequate renal perfusion.

Congestive heart failure (CHF) is a clinical situation 
associated with increased activity of vasoconstrictor 
and compensatory activation of systemic and intrarenal vasodilatory systems (6). It is 
 presently recognized that increased renal synthesis of 
vasodilatory PGs, in particular PGE_2 and PGI_2, is of primary importance to the maintenance of renal func-
tion in CHF (7, 23, 25). Consequently, blockade of PG 
synthesis in patients with CHF may result in deterio-
ration of renal function and precipitation of renal fail-
ure (7, 23). Similar observations have been reported in 
 studies in animal models of CHF, which further indi-
cated that inhibition of PG synthesis may be associated 
with adverse renal hemodynamic consequences (22, 
25). The cellular and molecular mechanisms underlying 
the increased synthesis of renal PGs in CHF, as 
well as the relative contribution of the two isoforms of 
COX, are largely unknown. This issue is pertinent, in 
view of the demonstration that the kidney contains 
both isoforms of COX, as well as the recent progress in 
development of selective blockers of these isoenzymes. 
Therefore, the present study was undertaken to exam-
ine the regulation of COX isoforms and the renal pro-
duction of PGs in rats with aortocaval fistula, an 
experimental model of CHF. Previously we have shown 
that this experimental model is characterized by 
changes in neurohormonal profile and in renal hemo-
dynamics that closely mimic the alterations in patients 
with severe CHF (1, 33). Accordingly, the expression of 
COX-1 and COX-2, in the cortical and medullary tis-
sues of rats with aortocaval fistula, and their immuno-
reactive protein levels were determined by RT-PCR 
and Western blotting, respectively. In addition, we 
evaluated the renal hemodynamic response to selective 
and nonselective blockade of the two COX isoforms.

MATERIALS AND METHODS

Studies were conducted on male Wistar rats of local strain, 
weighing 260–350 g. The animals were kept in individual

metabolic cages, in a controlled-temperature room, and were 
fed standard rat chow containing 0.5% NaCl and tap water ad libitum.

The Experimental Model

An aortocaval fistula was surgically created between the 
avdominal aorta and inferior vena cava according to the 
method originally described by Stumpe et al. (27) and 
adapted in our laboratory (1, 14, 33). Briefly, a midline 
avdominal incision was performed under pentobarbital 
sodium (Nembutal) anesthesia to expose the vena cava and 
avdominal aorta distal to the origin of the renal arteries. A 
longitudinal incision was performed in the outer wall of 
the vena cava. Under binocular magnification, the common wall 
between the aorta and vena cava was grasped through an 
incision, and a fistula (1.0–1.2 mm OD) was created between the 
the two vessels. The opening of the outer wall of the vena 
cava was closed with a continuous suture (7–0 prolene non-
absorbable suture, Ethicon). After the surgical procedure, the 
animals were allowed to recover and then were returned to 
the metabolic cages for daily monitoring of urine output and 
sodium excretion. A matched group of sham-operated rats 
served as controls. Six to seven days after the operation, rats 
with aortocaval fistula (ACF) were divided into two sub-
groups according to their daily absolute rate of sodium ex-
cretion (U_NaV <100 μmol/24 h) and rats with compensated CHF (U_NaV 
>1,200 μmol/24 h).

In Vitro Studies

To follow the alterations in gene expression and immuno-
reactive levels of COX-1 and COX-2 during the development of 
CHF, the following methodologies were used. Control rats 
and animals with compensated and decompensated CHF 
were decapitated, and their kidneys were removed and im-
mediately placed in liquid nitrogen. Total RNA was extracted 
from the renal cortex and medulla as described by Chromc-
zynski and Sacchi (5) with the use of a commercial solution 
(RNAzol B, Tel-Test) and was quantified by spectrophotom-
etry.

\[ \text{RT-PCR} \]

mRNA levels of COX-1 and COX-2 in the renal 
cortex and medulla were determined in control rats, and 
in rats with compensated and decompensated CHF (5–7 
animals/group). cDNA for these mRNAs were synthesized 
in rats with compensated and decompensated CHF 
by size PCR products derived from cDNA from those derived 
by 32 PCR cycles for COX-1 and 36 cycles for COX-2

RT-PCR. mRNA levels of COX-1 and COX-2 in the renal 
cortex and medulla were determined in control rats, and 
in rats with compensated and decompensated CHF (5–7 
animals/group). cDNA for these mRNAs were synthesized 
from 2 μg of total RNA with the use of specific primers, as 
shown in Table 1. In some experiments, additional up- 
and downstream primers for COX-2 were also used (upstream: 
5'-ACACTCTATCATGGCATCC-3' (bp 1229–1248); 
downstream: 5'-GAAGGGACACCCTTTCACAT-3' (bp 1794–1813); 
Genosys Biotechnologies). 

Avian myeloblastosis virus RT (AMV-RT, 16 U/reaction; 
Promega) was used for RT, along with the reaction mixture 
recommended by the enzyme manufacturer, in a volume of 20 
μl, using 2-μM downstream primer. PCR was then applied 
with 2 μl of the resulting cDNA using 1.25 μM of both the 
upstream and the downstream primers. Each PCR reaction 
mixture contained 1.5 U of the enzyme Taq 
polymerase (Takara Ex Taq, 16 U/reaction; 
Promega) was used for RT, along with the reaction mixture 
recomm
Table 1. Oligonucleotide primers for PCR used in the study

<table>
<thead>
<tr>
<th>Primer Sequences</th>
<th>Size, bp</th>
</tr>
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<tbody>
<tr>
<td>COX-1 (Accession no. U18060)</td>
<td>5’-CTGGCCGGATTGGGAAAATT-3’</td>
</tr>
<tr>
<td>Upstream</td>
<td></td>
</tr>
<tr>
<td>Bases</td>
<td>1298–1319</td>
</tr>
<tr>
<td>Downstream</td>
<td>5’-CATAGGGGCGAGTCTTGGTGG-3’</td>
</tr>
<tr>
<td>Bases</td>
<td>1716–1738</td>
</tr>
<tr>
<td>COX-2 (Accession no. L20085)</td>
<td>5’-ACTTCGTATGTTGCTGCTT-3’</td>
</tr>
<tr>
<td>Upstream</td>
<td></td>
</tr>
<tr>
<td>Bases</td>
<td>1465–1485</td>
</tr>
<tr>
<td>Downstream</td>
<td>5’-GACTACCTCATGAAGATCTGACC-3’</td>
</tr>
<tr>
<td>Bases</td>
<td>1723–1746</td>
</tr>
<tr>
<td>β-Actin</td>
<td>5’-TGATCTTCTATGGTGCTAGGAGCC-3’</td>
</tr>
<tr>
<td>Nucleotides encoding amino acids 320–327</td>
<td></td>
</tr>
<tr>
<td>Bases</td>
<td>430–435</td>
</tr>
</tbody>
</table>

COX-1, cyclooxygenase 1; COX-2, cyclooxygenase 2.

(for COX-2) for 45 s, elongation at 72°C for 1.5 min, and denaturation at 94°C for 45 s were performed with 2 μl of the cDNA as described above. The RT-PCR product of the gene encoding β-actin served as a quantity control, and it required 23 cycles to obtain a visible product. Six microliters of PCR products were electrophoresed on a 2.0% agarose-Tris-acetate-EDTA gel. The resulting gel was stained with ethidium bromide (0.5 μg/ml) for 15 min to produce clear, visible bands in ultraviolet light.

Determination of COX-1 and COX-2 immunoreactive levels by using Western blot analysis. Renal cortex and medulla of control, compensated CHF, and decompensated CHF rats were removed as described above, and tissues were then homogenized with a polytron homogenizer (M. Zippener, Staufen, Germany) in 2.5 ml of 10-mM sodium phosphate buffer, pH 7.4, containing 1 mM MgCl₂, 30 mM NaCl, 0.02% sodium azide, and a protease inhibitor cocktail tablet (Boehringer Mannheim). The homogenates were stored at −70°C until assayed. Kaleidoscope prestained standard molecular markers (Bio-Rad) were used for determination of the molecular weight of the immunoreactive products. Ten microliters of the tissue homogenates (100 μg protein) were treated with 10 μl sample buffer (10% SDS, 50% glycerol, 1 M Tris, 0.1% bromphenol blue, and 1 M dithiothreitol, pH 6.8) and placed in a boiling-water bath for 5 min. Samples were then electrophoresed on polyacrilamide, Tris-glycine gels 4–20% (Bio-Rad) in a boiling-water bath for 5 min. Samples were then electrophoresed on polyacrilamide, Tris-glycine gels 4–20% (Bio-Rad), and transferred electrophoretically to a nitrocellulose membrane (75 mA/gel for 60–90 min). The blots were blocked in 3% (wt/vol) nonfat dry milk in Tris-buffered saline (TBS) and 0.1% Tween 20 overnight. The nitrocellulose membranes were incubated for 2 h with 250-fold diluted monoclonal antibodies against COX-1 (Alexis) or COX-2 (Transduction Laboratories, Lexington, KY), then washed four times with TBS for 5 min each. After washing, the blots were incubated with 5,000–10,000-fold diluted peroxidase-conjugated rabbit anti-mouse IgG (Sigma) in TBS containing 0.1% Tween 20 for 60 min. Immunoreactive bands were visualized by the chemiluminescence detection system (Zymed). Negative controls were treated with nonspecific immune serum instead of primary antibody and processed simultaneously. All sections were counterstained with hematoxylin, and representative photographs were taken.

In Vivo Protocols

Measurements of intrarenal hemodynamics. On the day of the experiment, rats were anesthetized with inactin (thiobutabarbital sodium salt, RBI, Natick, MA; 100 mg/kg ip) and prepared for hemodynamic studies. The rats were placed on a temperature-regulated table (37°C), and their body temperature was continuously monitored. After tracheostomy, polyethylene catheters (PE-50, Portex) were inserted into the left carotid artery, right jugular vein, and bladder. Mean arterial pressure (MAP) was measured through the carotid arterial line by using a pressure transducer. A solution of 0.9% saline was infused intravenously, throughout the experiment, at a rate 1.5% body wt/h. The left kidney was exposed through a midabdominal incision and placed on a Plexiglas holder. Cortical blood flow (CBF) and MBF were measured simultaneously by laser Doppler flowmetry (model 4001, dual channel; Master Perimed) as previously described (12). For measurement of CBF, the probe was placed perpendicularly to the surface of the cortex, and MBF was measured by a probe inserted into the outer medulla at a depth of 4–5 mm. After an equilibration period, baseline recordings of 30 s were obtained at 5-min intervals for 30 min. Either piroxicam (COX-1 inhibitor), nimesulide (COX-2 inhibitor), or indo-methacin (nonselective COX inhibitor) (29) (all purchased from Sigma), dissolved in PBS containing Tween 80, was then administered intravenously at a dose of 5 mg/kg over a 5-min period to control rats (n = 6) and rats with either compensated or decompensated CHF (n = 5–6). Hemodynamic recordings were obtained for an additional 30 min after the injection of the drug.

Statistical Analysis

Evaluation of the data obtained in the in vitro studies in control, compensated CHF, and decompensated CHF was done by one-way ANOVA, followed by Dunnett’s test. In the in vivo studies, one-way ANOVA for repeated measures was utilized to evaluate the differences between the time points and baseline value in each group. Two-way ANOVA was used to compare the lines representing the various experimental
groups. \( P \leq 0.05 \) was considered statistically significant. Data are presented as means ± SE.

**RESULTS**

**In Vitro Studies**

**RT-PCR data.** Figure 1A shows the PCR products of representative agarose gels for COX-1, COX-2, and \( \beta \)-actin in the renal cortex and medulla of control rats and animals with compensated and decompensated CHF. Figure 1, B and C, summarizes the results obtained by computerized densitometry of the gel photographs of medullary COX-1 and COX-2, respectively (\( n = 5 – 7 \) for each parameter).

The size of the RT-PCR products was as predicted from published sequences of rat cyclooxygenases and \( \beta \)-actin (Table 1). When the PCR was performed in the absence of RT or RNA, these products were not observed, indicating that this reaction was highly specific and that the obtained products were derived from the mRNA and not the genomic DNA. The optimal number of amplification cycles used in the present study was chosen on the basis of preliminary experiments which showed a linear correlation between the number of cycles and the yield of PCR products of COX-1, COX-2 (in the renal medulla), and \( \beta \)-actin. Also, the amount of cDNA was within the linear range of cDNA amplification. Therefore, our RT-PCR conditions could be used for semiquantitation of the respective mRNA.

After 32 PCR cycles, COX-1 mRNA levels were detected only in the renal medulla of all groups (Fig. 1A). Induction of CHF did not alter the expression of COX-1 gene in the renal medulla in either compensated or decompensated rats (Fig. 1B). Compared with COX-1, 36 PCR cycles of amplification were required to observe a major COX-2 mRNA product within the linear range of amplification, suggesting a higher baseline expression of COX-1 compared with that of COX-2 in the renal medulla. Similar to COX-1, after 36 PCR cycles the expression of COX-2 was detected only in the medulla of all experimental groups, but not in the cortical tissue (Fig. 1A). Interestingly, the expression of COX-2 mRNA increased in proportion to the severity of myo-

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**Fig. 1.** A: representative agarose gels of RT-PCR-amplified cyclooxygenase-1 (COX-1), COX-2, and \( \beta \)-actin cDNAs. Specific cDNAs were transcribed from total RNA extracted from renal cortex and medulla of control rats and animals with compensated (Comp) and decompensated (Decomp) congestive heart failure (CHF). Nos. at right are the predicted sizes (in bp) for amplified products. For COX-1, 32 cycles were required to obtain a visible product, and 36 and 23 PCR cycles for COX-2 and \( \beta \)-actin, respectively. B: densometric analysis of COX-1 mRNA in renal medulla of control rats and animals with compensated and decompensated CHF. C: densometric analysis of COX-2 mRNA in renal medulla of control animals and rats with compensated and decompensated CHF. *\( P < 0.05 \) vs. controls.
cardial dysfunction, in particular in the decompensated CHF subgroup ($\Delta = 67 \pm 32\%$, $P < 0.05$, Fig. 1C).

**COX-1 and COX-2 immunoreactive levels.** A protein fraction of $\sim 70$ kDa was clearly recognized in the renal medulla and to a lesser extent in the cortex, after the incubation of cortical and medullary homogenates with anti-COX-1 antibodies under denaturing and reducing conditions (Fig. 2A). In parallel with our findings with RT-PCR, the immunoreactive levels of COX-1 in the medulla were comparable in sham-operated animals and rats with either compensated or decompensated CHF (Fig. 2A). The results of COX-2 obtained with Western blot analysis corresponded well with those of COX-2-specific RT-PCR. COX-2 immunoreactivity was not detected in the cortical homogenates of controls as well as CHF rats (Fig. 2A). However, substantial immunoreactive levels of COX-2 were detected in the medullary tissues of all groups and were significantly increased in the decompensated rats subgroup ($\Delta = 35 \pm 8\%$, $P < 0.01$, Fig. 2C).

**Immunohistochemistry.** Intense staining of immunoreactive COX-1 was detected in all portions of collecting tubules, especially in the epithelial cells of outer and inner medullary collecting duct, of normal rats and those with compensated and decompensated CHF (Fig. 3). A faint staining of COX-2 was also observed in some tubules of medullary thick ascending limb of Henle (data not shown). COX-1 immunoreactivity was non-detectable in the glomeruli and proximal tubules of these groups. In the renal cortex of all experimental groups, an intense COX-2 immunoreactivity was detected in the macula densa and its containing segment (Fig. 4) but not in glomeruli, in agreement with previous reports (31). COX-2 immunostaining was low in the cortex; however, high COX-2 immunoreactivities were revealed in the epithelial cells of outer and inner medullary collecting duct of all groups (Fig. 5). Although only roughly quantitative, immunohistochemical analysis indicated that a stronger staining was detected in the outer and inner medulla of animals with CHF, especially those with a decompensated disease. It should be emphasized that high basal levels of COX-2 are present in the macula densa and renal medulla of normal rats, confirming previous studies that the kidney is one of the tissues where COX-2 is constitutively expressed.

**Fig. 2.** A: Western blot analysis of COX-1 and COX-2 in renal cortex and medulla of control rats and rats with compensated and decompensated CHF. Immunoreactive bands were visualized with an enhanced chemiluminescence detection system (Sigma). Macrophage cell lysate served as a positive control for COX-2. Bands at $\sim 70$ kDa represent COX-1 and COX-2 immunoreactive proteins. B: densitometric analysis of COX-1 in renal medulla of control rats and animals with compensated and decompensated CHF. C: densitometric analysis of COX-2 in renal medulla of control rats and rats with compensated and decompensated CHF. $*P < 0.01$ vs. controls.
**In Vivo Protocols**

**Effects of COX inhibitors on intrarenal hemodynamics.** In line with our previous report (1), there was a significant decrease (by $-30\%$) in baseline CBF in rats with CHF compared with control animals $[207 \pm 5.8$ vs. $297 \pm 5.8$ perfusion units (PU), $P < 0.05]$, whereas MBF was largely preserved $[80 \pm 1.4$ vs. $79.5 \pm 1.7$ PU, $P = \text{not significant}]$.

Acute administration of 5 mg/kg of either piroxicam, nimesulide, or indomethacin did not produce any consistent change in CBF, either in sham-operated rats or in rats with CHF, for the 30-min observation period after drug administration (data not shown). Similarly, CBF was unchanged throughout the experiment, in controls or in rats with CHF, by the administration of the vehicle alone. In contrast to its effect on the CBF, indomethacin provoked a significant and sustained decrease in MBF ($\Delta = -20\%$). Similar responses in magnitude and duration were obtained after the administration of the selective COX-1 inhibitor, i.e., piroxicam, and the selective COX-2 inhibitor, i.e., nimesulide (Fig. 6A).

In a few preliminary studies, administration of NS-398, a selective inhibitor of COX-2, at a dose of 3 mg/kg decreased MBF by 10–13% without affecting CBF (data not shown). In rats with CHF, the effect of nimesulide on MBF tended to be more sustained than that of piroxicam, whereas the most pronounced reduction in MBF was observed after indomethacin administration (Fig. 6B). Administration of vehicle alone did not alter MBF in both control rats and rats with CHF. The effect of indomethacin

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Fig. 3. Paraformaldehyde-fixed, paraffin-embedded sections of renal medulla stained by COX-1 antibody or by nonspecific immune serum (A) from sham-operated rats (B) and from rats with compensated (C) and decompensated CHF (D).
on MBF was significantly different \( (P < 0.003, \) by 2-way ANOVA) from the lines representing either piroxicam or nimesulide.

**DISCUSSION**

The present study provides novel information on the regulation of renal PG synthesis in rats with aortocaval fistula, an experimental model of CHF. Our findings suggest that the COX-2 isoform, rather than COX-1, is upregulated in the kidney of rats with experimental heart failure, in proportion to the severity of the disease. Moreover, this change occurs primarily in the renal medulla, a region where COX-2 is present constitutively in high abundance. Finally, our study suggests that this increased expression of COX-2 may play a significant role in the maintenance of medullary perfusion in rats with experimental CHF, as depicted by the changes in MBF in response to administration of selective inhibitors of the COX isoforms.

It is presently recognized that CHF is associated with increased synthesis of PGs, in particular PGE\(_2\) and PGI\(_2\), as expressed by the elevated levels of these agents and their metabolites in the plasma and urine of patients and animals with myocardial dysfunction \((7, 8)\). Although the importance of the vasodilatory PGs in maintaining RBF in heart failure is well recognized,
the mechanisms responsible for their increased synthesis have not been yet fully elucidated. The findings of the present study suggest that transcriptional upregulation of the COX-2 isoform in the kidney may be an important factor in the generation of vasodilatory PGs in rats with experimental heart failure, in particular in the renal medulla. Indeed, both RT-PCR and Western blot data in our study revealed that both isoforms of COX are abundantly expressed in the renal medulla, at a level that is significantly higher than in the cortex. Similar results were reported by several groups, who demonstrated that high levels of COX-2 mRNA are present in the renal medulla, mainly in the medullary interstitial cells (11, 13, 36). Taken together, these results suggest that the renal medulla is a major site of COX-2 expression where, together with COX-1, both isoforms mediate PG generation. Because COX-2 and COX-1 share similar catalytic properties, it might be assumed that both isoforms contribute significantly to the production of PGs in the kidney, particularly in the renal medulla. These findings on the expression of both isoforms of COX in the renal medulla are consistent with previous reports suggesting that the medulla is a major site of PG synthesis in the kidney, significantly exceeding that of the cortex (9, 32). In contrast, the synthesis of PGs in the cortex appears to be lower compared with the medulla, corresponding to the negligible expression and immunoreactivity of both isoforms of COX. The highly localized expression and immunoreactive protein of COX-2 in the macula densa
and surrounding region in the renal cortex (Fig. 4) is in line with the low levels of this isoform in the whole cortical tissue. Similarly, recent studies have demonstrated that COX-2 is constitutively present in the macula densa segment of the rat kidney (13, 36), suggesting a potential role for this isoform in the juxtaglomerular apparatus-mediated tubuloglomerular feedback (TGF) response and renin secretion. In this context, Ichihara et al. (16) have demonstrated that, during increased activation of TGF-dependent vasoconstriction signals, COX-2 generates vasodilatory metabolites and thus participates in the counteracting modulation of TGF-mediated afferent arteriole constriction. Hence, changes circumscribed to small portions of the renal tissue, such as the macula densa or the afferent arterioles, could not be detected by our methodology, which is more suitable for detection of changes in bulk COX-1 and COX-2 expression.

The finding that both isoforms of COX are highly expressed in the renal medulla and that COX-2 is further upregulated in rats with severe heart failure suggests that this phenomenon might be of pathophysiological importance. Previously we have shown that medullary perfusion is maintained in rats with experimental CHF despite a marked degree of cortical vasoconstriction (1). The preservation of MBF in the face of a decrease in renal perfusion pressure, such as occurs in this situation, suggests that medullary circulation is under a tonic vasodilatory control. Indeed, we have demonstrated that endothelial nitric oxide synthase is predominantly expressed in the medulla and that its expression is further increased in proportion to the severity of heart failure. This suggests that experimental CHF is associated with an adaptive increase in the expression of the nitric oxide (NO) system in the kidney and that enhanced NO activity in the medulla contributes to the preservation of MBF. The present study provides evidence that decompensated CHF is also associated with enhanced expression of COX-2 and, presumably, enhanced production of vasodilatory PGs in the renal medulla, which, like the NO system, may contribute to the preservation of MBF. Thus the two vasodilatory systems act in concert to maintain adequate blood supply to the medulla when renal perfusion pressure is decreased due to cardiac failure.

The importance of PG generation in the renal medulla is further underscored by the findings of the in vivo experiments, which evaluated the changes in regional RBF in response to selective and nonselective inhibition of the COX isoforms. Our study did not disclose any consistent change in CBF after COX inhibition, either in control rats or in rats with experimental CHF. In contrast, the same doses of COX inhibitors produced a significant and sustained reduction in MBF in both control and CHF rats. Furthermore, although in control rats administration of either selective or nonselective COX inhibitors produced a comparable decrease in MBF, the effect of the nonselective COX inhibitor in rats with CHF was greater than that observed with either selective COX-1 or COX-2 inhibitors. This might suggest that in rats with CHF both isoforms contributed significantly to the preservation of MBF and that combined inhibition of the isoforms is required to reach a maximal effect. Our findings regarding the high vulnerability of the medullary blood supply to acute inhibition of COX, with no change in CBF, are consistent with previous publications utilizing similar methodologies (2, 19, 26). The lack of change in CBF in response to COX inhibition in either normal or CHF rats in the present study does not rule out the possibility that PGs are involved in the regulation of blood supply to the cortex. Rather, it is possible that a longer period of observation (greater than the 30 min utilized in present study) or chronic treatment is required before such an effect might become noticeable.

The mechanism responsible for the upregulation of the COX-2 in the present study remains to be elucidated. An interesting possibility is that the increase in COX-2 is related to the activation of the NO system in the renal medulla. Thus it has been demonstrated in rat osteoblasts that NO can induce PG synthesis and COX-2 expression and may regulate the expression of this enzyme at both transcriptional and posttranscrip-
tional levels (15). Furthermore, NO has been shown to amplify the stimulatory effect of cytokines on COX-2 expression in rat mesangial cells (28). Further studies are required to elucidate this interaction in our model. It has also been suggested the local production of PG in CHF is stimulated by tissue ischemia as well as by the direct influence of vasoactive substances, such as ANG II (7). In this respect, studies in patients with CHF have demonstrated a direct linear relationship between plasma renin activity and ANG II concentrations and the circulating levels of PGE₂ metabolites, suggesting a stimulation of PG synthesis by ANG II (8).

Indeed, the experimental model utilized in the present study, rats with arteriovenous fistula, is characterized by increased activity of the renin-angiotensin axis, in proportion to the severity of the disease (24, 33). However, the possibility that ANG II may trigger renal PG synthesis by upregulation of COX-2 is not supported by the recent study of Cheng et al. (4), who demonstrated that inhibition of ANG II in rats with volume depletion, by either an angiotensin-converting enzyme inhibitor or an ANG II-receptor antagonist, resulted in augmentation rather than suppression of cortical COX-2 expression, suggesting an inhibitory relationship between ANG II and COX-2. Unfortunately, their study did not examine specifically the expression of COX-2 in the medulla, the major site of expression of this isoform in the kidney. Such determination is of special interest in light of the possible divergent regulation of COX-2 in the cortex and medulla. For instance, after chronic salt depletion, COX-2 expression in the peri-macula densa region increased significantly, although medullary interstitial cell expression decreased (17, 36). On the other hand, in rats on a high-salt diet the expression of COX-2 in the inner medulla increased, whereas cortical COX-2 levels decreased. Interestingly, the expression of COX-1 in the medullary and cortical tissues was not affected by dietary salt intake (36). These findings, like our results that medullary COX-1 expression and immunoreactivity were unchanged by the induction of CHF, are consistent with the notion that COX-1 fulfills “housekeeping” functions and is less subject to strict regulation. This, however, does not exclude an important contribution of this isoform to renal PG synthesis in CHF or the possibility that regulation of COX-1 may occur at a posttranscriptional level. In contrast, the changes in medullary COX-2 in rats with experimental CHF as observed in our study or in response to high dietary sodium (36), as well as the increase in glomerular COX-2 in the remnant kidney in the renal ablation model (31), suggest that COX-2 is subject to regulation by factor(s) related to volume and salt balance. It appears that such a regulation of COX-2 in the kidney is not unique to CHF, but may also occur in other volume expansion conditions where the augmented PG production provokes the elimination of the excess of salt and fluids, thereby protecting against volume overload.

In summary, our study demonstrates that although both isoforms of COX are highly expressed in the renal medulla, only the COX-2 isoform is upregulated in rats with experimental CHF, in proportion to the severity of the disease. The findings further suggest that this increase in COX-2 may play an important role, presumably with other local vasoactive systems, in the preservation of blood supply to the renal medulla during the development of cardiac failure.

The authors are grateful to Felix Milman for expert technical support and to Ruth Singer for secretarial assistance. This study was supported by Israeli Ministry of Health Grant 4644 (to J. Winaver).

REFERENCES


