Tubuloglomerular feedback is decreased in COX-1 knockout mice after chronic angiotensin II infusion

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Araujo M, Welch WJ. Tubuloglomerular feedback is decreased in COX-1 knockout mice after chronic angiotensin II infusion. Am J Physiol Renal Physiol 298: F1059–F1063, 2010. First published January 27, 2010; doi:10.1152/ajprenal.00547.2009.—Prostaglandins (PGs), produced by two isoforms of cyclooxygenase (COX), COX-1 and COX-2, are important modulators of renal hemodynamics. COX-1 and COX-2 are expressed in the kidney often at distinct sites. Thromboxane (TxA2), PGE2, and prostacyclin (PGI2) are the major PGs in the renal cortex of mice. Acute infusion of the vasoconstrictor ANG II increases COX-2-dependent PGE2 and PGI2. COX-2 is primarily expressed in the macula densa (MD), where several PG synthases are also expressed. We previously showed that MD COX-2 products modulate tubuloglomerular feedback (TGF) in the rat. Genetic deletion of COX-1 enhances COX-2 production of PGs, decreases renal and urinary PGs, and attenuates ANG II-induced hypertension. The present study tested the effects of chronic ANG II infusion on TGF in COX-1 knockout (KO) mice. Basal TGF was similar in COX-1 KO and wild-type (WT) mice. Chronic ANG II infusion increased TGF in WT mice (WT: 9.3 ± 0.7 vs. WT + ANG II: 12.2 ± 1.6 mmHg, P < 0.02). However, chronic ANG II decreased TGF in COX-1 KO mice (KO: 11.4 ± 1.1 vs. KO + ANG II: 8.3 ± 0.6 mmHg, P < 0.01). Pretreatment with the COX-2 inhibitor SC-58,236 in COX-1 KO mice prevented the ANG II-associated reduction in TGF (11.4 ± 1.0 vs. 11.5 ± 0.28 mmHg, not significant). Excretion of 6-keto-PGF1α, the metabolite of PGI2, was increased by ANG II infusion, whereas excretion of TxB2, the stable metabolite of TxA2, was not changed. ANG II infusion increased mean arterial pressure similarly in both WT and KO mice (WT: 93 ± 2 vs. KO: 92 ± 3 mmHg), but not in KO mice pretreated with SC-58,236 (85 ± 2 mmHg). This study shows that COX-1-generated PGs partially mediate ANG II increases in TGF and that COX-2 PGs offset that effect.

Cyclooxygenase; prostaglandins; hypertension

CYCLOOXYGENASE (COX) acts on arachidonic acid to generate endoperoxides (PGH2), which are metabolized to form multiple prostaglandins (PGs) by various PG synthases (PG-S). PGs act as autacoids in multiple tissues and many are vasoactive. In the kidney, PGs modulate hemodynamics, renin release, and tubular salt and water homeostasis (2, 6, 7, 18). Thromboxane (TxA2), prostaglandin E2 (PGE2), and prostacyclin (PGI2) are vasoactive PGs produced in the kidney. Therefore, the delivery and efficacy of these PGs are heavily dependent on colocalization of COX and the specific synthases. In the mouse kidney, COX-1 is expressed in the glomerulus, mesangial cells, cortical and outer medullary interstitial cells, late distal convoluted tubule, connecting tubule, and collecting duct principal cells (2). COX-2 is constitutively expressed in the macula densa (MD) and MD region in the mouse kidney, but not at the adjacent thick ascending limb, as in the rat kidney (2, 6).

Clinical and animal studies indicate that COX-1 and COX-2 activity differentially influences renal and cardiovascular function (4, 12, 16, 17). Since renal COX-1 is more broadly expressed than COX-2 and is more abundant, it is responsible for basal production of PGs. Whereas COX-2 is induced, it does contribute to basal PGs production in the renal medulla (4, 16, 17, 25). COX-2 in the MD generates PGs that may play critical roles in regulation of renin release and control of afferent arteriolar tone necessary for tubuloglomerular feedback (TGF) (7). TGF is a regulatory mechanism that acts at the single nephron level to stabilize glomerular filtration rate (GFR). During conditions that increase GFR, TGF is initiated and reduces GFR by release of mediators from the MD that constricts the adjacent afferent arteriole (20, 22). TGF is modulated by multiple autacoids and hormones including ATP, angiotensin II (ANG II), nitric oxide (NO), and the vasoactive PGs: PGE2, PGI2, and TxA2 (19, 21–23). TGF is enhanced in augmented ANG II conditions, such as one-kidney, one-clip hypertensive rats (15, 19), in the nonclipped kidney of Goldblatt hypertensive rats (8), and during suppressor doses of ANG II in rats (14).

However, the direct influence of renal COX-1 and COX-2 on TGF modulation during ANG II-dependent hypertension remains uncertain. The present study was designed to evaluate whether vasoactive COX products mediate the effects of chronic infusion of ANG II on TGF. We tested the hypothesis that TGF is enhanced during low-dose ANG II infusion by local release or generation of vasoconstrictor PGs. We measured TGF in wild-type (WT) and COX-1 knockout (KO) mice after 2-wk infusion of ANG II or vehicle.

MATERIALS AND METHODS

Animals. Three to 4-mo-old male COX-1 WT (+/+) and KO (−/−) mice weighing 23–28 g were housed in a quiet room at 25°C with a 12:12-h light-dark cycle with free access to food and water. Animals were obtained from established colonies at the Georgetown University. The generation and genotyping of COX-1 gene-deficient mice (C57/B16 × 129 background) have been previously reported (9).

This study was approved by the Georgetown University Animal Care and Use Committee and performed according to the National Institutes of Health guidelines for the conduct of experiments in animals.

Induction of slow pressure ANG II hypertension. Hypertension was achieved after 2 wk of infusion of ANG II at 400 ng·kg−1·min−1 that is initially a suppressor, but increases mean arterial pressure (MAP) after 5–7 days (19). We implanted osmotic minipumps subcutaneously (model 1002; Alzet, Palo Alto, CA) containing ANG II dissolved in sterile saline or saline alone (Vehicle). Minipumps were implanted in COX-1 (+/+) and COX-1 (−/−) mice during isoflurane anesthesia (2%). Animals were allowed to recover and monitored daily.

TGF experiments. For surgical preparation for acute measurements of TGF, mice were anesthetized with isoflurane (2% in room air) and placed on a servo-controlled heating table to keep body temperature at
37°C. The trachea was cannulated and immediately connected to a gas anesthesia system (400 Anesthesia Unit, Univentor) where a mixture of 2% isoflurane with room air was constantly infused throughout the surgery. Catheters were placed in the left jugular vein for fluid infusion and in the right femoral artery for recording of MAP from the electrically damped output of a pressure transducer. The left kidney was exposed by a flank incision, cleaned of connective tissue, and stabilized in a Lucite cup. This kidney was bathed in 0.154 M NaCl solution maintained at 37°C. The bladder was cannulated to measure urine volume. After completion of surgery, the isoflurane was decreased to 1.5% and kept constant throughout the experiment. Mice received 0.1 ml (bolus, iv) of 0.154 M NaCl solution containing 1.5% bovine serum albumin (Sigma, St. Louis, MO), followed by constant infusion at 0.35 ml/h to maintain euvo1emia. Micropuncture studies were initiated after 45 min and performed as previously described in rats (22). Briefly, a micropipette (8-μm OD) containing artificial tubular fluid (ATF; in mM: 123 NaCl, 4 NaHCO3, 5 KCl, 2 CaCl2, 7 urea, 2 MgCl2) stained with FD&C # 2 dye was inserted into a proximal tubule to identify the nephron and the direction of the flow. Subsequently, a grease block (T grease, Apiezon, Manchester, UK) was inserted into the micropuncture site with a micropipette (8-μm OD) connected to a hydraulic drive (Trent Wells, La Jolla, CA) to halt tubular flow. A perfusion pipette containing ATF plus dye was inserted into the late proximal tubule downstream from the grease block and connected to a nanoliter microperefusion pump (Vestavia Scientific, Birmingham, AL). A pressure pipette (2-to-3-μm OD) was inserted into the proximal tubule upstream from the grease block to measure proximal stop-flow pressure (Psf). The pressure was recorded in a micro pressure system (model 900A, World Precision Instruments, Sarasota, FL) connected to a Powerlab system (AD Instruments, Colorado Springs, CO).

Psf was recorded in each nephron at zero loop perfusion (2 min) and during perfusion at 10 nl/min (1 min) and 30 nl/min (2 min). This strategy was used to avoid leakage of the fluid when rate was changed from 0 to 30 nl/min, which produces a maximal TGF response. Maximal TGF responses were calculated from average Psf obtained from 0 to 30 nl/min, which produces a maximal TGF response.

**Results**

Urine flow was similar in all groups (Table 1). MAP under anesthesia was similar in normal WT and KO mice. Infusion of slow-pressor doses of ANG II for 2 wk increased blood pressure in both WT and KO mice (P < 0.01; Table 1). However, ANG II did not increase MAP in KO mice pretreated with SC-58,236 in MAP (Table 1).

Excretion of 6-keto-PGF1α and PGE2 was higher in COX-1 mice treated with ANG II (Fig. 1). However, excretion of TxB2 was not altered by ANG II. The PGL2 metabolite, 6-keto-PGF2α, was the predominant PG excreted after ANG II.

Figure 2 shows Psf in proximal tubules of COX-1 WT and KO mice with zero perfusion or during perfusion of loop of Henle with ATF at 30 nl/min. TGF, calculated from the difference in Psf at 0 and 30 nl/min perfusion, averaged 10–11 mmHg in both groups and was not different between groups.

The maximal TGF was increased in COX-1 WT mice infused with low-dose ANG II infusion for 2 wk (WT: 9.3 ± 0.7 vs. WT + ANG II: 12.2 ± 1.6 mmHg, P < 0.02; Fig. 3). Conversely, the maximal TGF was decreased in COX-1 KO mice treated with ANG II (KO: 11.4 ± 1.1 vs. KO + ANG II: 8.3 ± 0.6 mmHg, P < 0.01; Fig. 4). These data suggest that COX-1 contributes to normal maintenance of TGF and responds to ANG II stimulation.

We previously showed that TxA2 was nearly absent in COX-1 KO mice and that vasodilating PGs were partially preserved, presumably by COX-2 (18). Therefore, to understand the possible role of COX-2 in the suppression of TGF by ANG II in the COX-1 KO mice, we treated separate COX-1 KO mice with SC-58,236 before and simultaneously with ANG II. TGF was normalized in this group (11.5 ± 0.28 mmHg, not significant; Fig. 4). These data suggest that in the absence of COX-1, COX-2 produces primarily vasodilating PGs in response to ANG II, resulting in a lower TGF.

**Discussion**

We found that basal TGF was not affected by the genetic deletion of COX-1, yet the enhancement of TGF typically seen with systemic infusion of ANG II was abolished in COX-1 KO mice. The lack of any effect on TGF at baseline reflects the role of the kidney to maintain a stable GFR in the steady state. However, when the kidney is challenged by a slow-pressor infusion of ANG II, TGF is increased consistent with its effect as a vasoconstrictor. Yet, during ANG II infusion, TGF was actually decreased in COX-1 KO mice. This result suggests two possibilities: 1) COX-1 KO lack sufficient vasoconstrictor products that normally complement the ANG II effect and without this, TGF was diminished, or 2) COX-2 is upregulated.

**Table 1. MAP and V obtained during micropuncture studies**

<table>
<thead>
<tr>
<th>Condition</th>
<th>MAP, mmHg</th>
<th>V, μl/min</th>
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<tbody>
<tr>
<td>WT (n = 6)</td>
<td>82 ± 2</td>
<td>1.1 ± 0.1</td>
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<tr>
<td>WT + ANG II (n = 6)</td>
<td>93 ± 2*</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>KO (n = 6)</td>
<td>78 ± 2</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>KO + ANG II (n = 7)</td>
<td>92 ± 3*</td>
<td>1.5 ± 0.4</td>
</tr>
<tr>
<td>KO + ANG II + SC236 (n = 6)</td>
<td>85 ± 3</td>
<td>1.0 ± 0.4</td>
</tr>
</tbody>
</table>

Values are means ± SE. MAP, mean arterial pressure; V, urine output; WT, wild-type; KO, knockout. *P < 0.01 compared with nontreated mice by unpaired t-test and ANOVA.
or induced by ANG II and produced excessive vasodilator products that offset ANG II actions on TGF, greatly suppressing the maximal response. Previously, we showed that excretion of TxB2 and PGE2 was lower in COX-1\(^{-/-}\) mice, whereas the excretion of 6-keto-PGF\(_{2\alpha}\) was not different \((9)\). As shown in Fig. 1, infusion with ANG II in this study had no effect on TxB2 excretion in COX-1\(^{-/-}\) mice. However, ANG II increased 6-keto-PGF\(_{2\alpha}\) and PGE2 excretion in COX-1\(^{-/-}\) mice. These data show that PGI\(_2\) is preferentially generated in COX-1\(^{-/-}\) mice, presumably due to upregulated COX-2. In addition, after ANG II the profile of the excreted PGs shows that the vasodilator PGI\(_2\) dominates. Therefore, we suggest that this different profile of PGs influences the overall effect of PGs and shifts the balance toward vasodilation. This profile of PGs is responsible for the reduced ANG II effect on TGF.

To test the second possibility, we further showed that specific pharmacological blockade of COX-2 in the COX-1 KO mice prevented the reduced TGF response to the slow-pressor ANG II. This suggests that COX-2, which is expressed in the MD, generates vasodilator products in the COX-1 KO contributing to the reduced TGF in these mice. It is unclear whether this restoration of TGF by COX-2 inhibition contributed to the modest reduction of MAP with this agent. These results therefore implicate both COX isoforms in the regulation of TGF. Since COX-1 is expressed in several vascular sites, its products may act in concert with ANG II to constrict small resistance vessels. The effects of COX-1 products would not be apparent unless ANG II was elevated, presumably enhancing resistance in the afferent arterioles. The effects of COX-2, however, would arise from the MD in response to activation of TGF. Therefore, vasodilating PGs formed in the MD, such as PGI\(_2\) or PGE\(_2\), would offset TGF. Therefore, when COX-2 was suppressed by SC-58, 236 in the COX-1 KO mice, TGF was fully operational, without both COX-1 and COX-2 products. This once again demonstrates the multiple, almost redundant regulation of this important system controlling GFR. Even under conditions in which two important modulators of TGF are suppressed, this system relies on other vasoactive agents to stabilize TGF. These other possibilities are not explored in this study.
study, but include ANG II, adenosine, ATP, NO, and superoxide, among many others. Indeed, we previously showed that each of these systems can modulate or modulate TGF (22–24).

ANG II infusion promotes the synthesis of both constricting and dilating prostanoids, in vascular and renal tissues (5, 11, 13, 24). However, Qi et al. (16) showed that selective inhibition of COX-2 enhances the pressor effects of ANG II, further reducing renal medullary blood flow in mice. This suggests that ANG II also increases production of dilating PGs. Conversely, inhibition of COX-1 attenuates ANG II-induced hypertension (7). COX-1 inhibition also attenuates ANG II-dependent increase of cortical PGF<sub>2α</sub>, a potent vasoconstrictor that binds with high affinity on EP1 and EP3 receptors (16).

ANG II has reduced effects on blood pressure in thromboxane receptors (TP) KO mice and COX-1 KO mice compared with WT (5), implicating the important role of TP receptors on regulation of vascular tone. Our results are consistent with this study and further suggest that TGF is enhanced by chronic slow-pressor doses of ANG II through COX-1-derived vasoconstrictive PGs.

The fact that COX-1 (−/−) mice have normal TGF responses corroborates our previous findings in rats that MD COX-2 directly modulates TGF in steady-state conditions (1). Acute MD COX-2 inhibition attenuated TGF maximal responses mostly through TxA<sub>2</sub>, while acute COX-1 inhibition had only a minor effect on TGF (1). These two separate studies imply that the effects of acute and chronic inhibition of COX-1 and COX-2 on TGF may be different.

There is increasing evidence that ANG II modulates COX-2 expression through AT1 and AT2 receptors (3, 23, 26). A recent study shows that renal COX-2 expression is not changed in COX-1 KO mice (2). Our data suggest that ANG II acts on MD COX-2 to produce vasodilating prostanoids responsible for lowering TGF in the absence of COX-1. Whether this effect is due to a change of COX-2 expression or activity needs to be determined. MAP under anesthesia was similar in COX-1 WT and COX-1 KO after 2 wk of ANG II infusion. This would disagree with previous studies showing attenuated ANG II-dependent hypertension in COX-1 KO mice (5, 17). However, we did not measure MAP in conscious animals. The fact that MAP was not elevated in COX-1 KO mice treated with both SC-58,236 and ANG II could suggest that 1) in the absence of COX-1, ANG II induces high blood pressure through COX-2-vasoconstricting prostanoids or 2) the pretreatment with SC-58,236 for 1 wk decreased basal MAP of COX KO mice and the ANG II effect could not be detected at the endpoint.

We previously showed that thromboxane contributes to the vasconstriction induced by TGF (23). Therefore, part of the increased role of COX-2-derived PG<sub>D</sub> could be due to the diminished production of thromboxane. TxB<sub>2</sub> excretion in COX-1 KO mice is reduced by 90% (9) and we show in this study that TxB<sub>2</sub> excretion was not increased by ANG II treatment. Ultimately, this reflects on the balance between the PGs in this model, which is shifted toward dilating PGs.

In summary, low-dose chronic ANG II increases TGF partially via COX-1 products that are predominately vasoconstrictors. However, in the absence of COX-1, ANG II stimulates MD COX-2 products, which are primarily vasodilators, to decrease TGF. This is the first study that directly evaluates the role of COX-1 on TGF in normal conditions and during ANG II-dependent hypertension. It adds additional evidence to clarify the roles of COX-1 and COX-2 in the control of renal hemodynamics. These results also support the link of COX-1 to the renal vasoconstriction caused by NSAIDS in patients with hypertension. Future studies need to identify the specific PGs and PG-S produced in the MD, which may participate in TGF regulation. In conclusion, this study demonstrates that PGs generated by both COX isoforms regulate TGF.

DISCLOSURES

No conflicts of interest are declared by the authors.

REFERENCES


