Tetradecylthioacetic acid downregulates cyclooxygenase 2 in the renal cortex of two-kidney, one-clip hypertensive rats

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Bivol LM, Hultström M, Gudbrandsen OA, Berge RK, Iversen BM. Tetradecylthioacetic acid downregulates cyclooxygenase 2 in the renal cortex of two-kidney, one-clip hypertensive rats. Am J Physiol Regul Integr Comp Physiol 295: R1866–R1873, 2008. First published October 8, 2008; doi:10.1152/ajpregu.00850.2007.—The effect of tetradecylthioacetic acid (TTA) on the cyclooxygenase (COX) system was investigated in two-kidney, one-clip (2K1C) hypertensive rats. The systolic blood pressure (BP) was increased 6 wk after clipping to 183 ± 4 vs. 127 ± 3 mmHg in TTA-treated 2K1C rats. The COX1 protein expression was not affected either by the 2K1C procedure or by TTA treatment. COX2 expression was upregulated in both kidneys, but to a greater extent in the clipped kidney. COX2 activity was increased in 2K1C (31 ± 0.3 in the clipped and 28 ± 0.2 pg/ml nonclipped kidney, P < 0.001 compared with control). TTA lowered the PGE2 to control levels. Renal blood flow (RBF) response to exogenous ANG II injected in the control and nonclipped kidney was exaggerated after indomethacin treatment but unchanged in the nonclipped kidney of the K1C TTA group. Overall, these results indicate that, after 6 wk of treatment, TTA downregulated the COX2 activity, which have potentially important effects on the regulation of renal hemodynamics but does not explain TTA ability to lower BP.

Prostaglandings (PG) are involved in regulation of many processes in the human body (26, 47), and PGE2, a member of the PG family, is involved in hypertension, inflammation, (47) and, importantly, the regulation of kidney function (43, 47). The key enzymes in the generation of prostaglandins are the cyclooxygenases (COX) (47). Both COX1 and COX2 are constitutively present in the kidney cortex (19, 48, 53). In addition, COX2 is induced in 2K1C renovascular hypertension (19, 27), as well as in other models with high ANG II levels (24). The PG-E synthases (PGES) are downstream enzymes that produce PGE2, and COX2 may be more selectively linked to the terminal PGE2 synthase than COX1 (30, 45). Increased PGE2 production may thus indicate increased COX2 activity (36, 47, 57). Indeed, COX2-selective blockers reduce the levels of PGE2 and the inflammatory response (29, 55).

It has been shown that COX2 and renin expression have a parallel regulation (19, 21), and selective COX2 blockers have been shown to reduce renin activity (19). For example, in COX2-deficient mice, the plasma renin level is low with reduced response to acute stimulation (25). In addition, there are studies that have shown a reduction of blood pressure (BP) after treatment with COX2 blockers in both 2K1C hypertension and inducible ANG II-dependent malignant hypertension in CYP1A-Ren2 transgenic rats (35, 56), although other reports show no effect on BP after COX2 inhibition as shown in a recent study by Richter et al. (41).

Tetradecylthioacetic acid (TTA) is a modified fatty acid that cannot undergo β-oxidation (2), and it is known to exert its various effects (4) by peroxisome proliferator-activated receptor (PPAR)-dependent or -independent pathways (3, 14). We have recently shown that TTA prevents the development of hypertension in 2K1C by reducing the activation of the renin-angiotensin system (5, 16) and, furthermore, that TTA has a strong anti-inflammatory effect (6). TTA reduces mRNA renin levels in the clipped kidney and ANG II in both clipped and nonclipped kidney (16). Because COX2 is enhanced in high renin models and TTA reduces the activity of the renin-angiotensin system, we hypothesized that TTA would decrease COX2 activity and PGE2 production, mechanisms that may contribute to the BP-lowering effect in 2K1C hypertension.
**MATERIALS AND METHODS**

**Animals.** The study was performed in 60 Wistar male rats HanTae:WH M from Mollegaard Breeding Colony (Skensved, Denmark) with a body weight of 180–200 g at the beginning of the study. The experiments were performed in accordance with, and under the approval of, the Norwegian State Board for Biological Experiments, the Guide for the Care and Use of Laboratory Animals, and the Guidelines of the Animal Welfare Act. The rats were housed in cages with constant temperature (25°C) and humidity. They were exposed to a 12:12-h light-dark cycle (light from 0600 to 1800) and had unrestricted access to water and food.

**Induction of 2KIC hypertensio**n. The right kidney was exposed through a lumbar incision, and the right renal artery was dissected free and clipped by a rigid U-shaped silver clip with an internal opening of 0.25 mm. The animals were anesthetized by isoflurane anesthesia (1/1000 Foren; Abbott) mixed with 2 l/min O2 using an Ohmeda Isotec 3 anesthesia utility (BOC; Health Care). To reduce the pain following the surgery, the rats received 0.3 mg/kg Temgesic after the surgery and the next 2 days. The animals were followed for the next 6 wk until the renal hemodynamic studies were performed, or the collection of plasma, urine, and kidney was made.

**Measurements of BP.** Systolic BP was measured before clipping and weekly during the development of hypertension by means of the tail-cuff method (UGO BASILE) in unanesthetized animals. The rats were prewarmed to 35°C for 10 min in a cupboard before the measurements.

**The study groups.** The animals were randomized in four sets of experiments, each set consisting of the following three groups: one nonclipped control group and two groups with 2KIC hypertension, whereof one group was nontreated (2KIC) and one group was TTA-treated (2KIC TTA). The first set of experiments was used for collection of renal cortex and urine (n = 6 in each group). Because TTA do not affect BP in control rats, this group was not examined in the present study (5, 6). One urine sample per rat was collected, as well as one tissue sample from the left kidney in the control group and one sample from each kidney in the 2KIC group. The second set of experiments was used for renal hemodynamic study (n = 6 in each group). The third group was used for studies of PPARs in clipped and nonclipped kidneys with and without TTA treatment. Furthermore, one group was used to study the effect of a COX2 inhibitor on BP. The control and 2KIC hypertensive groups were fed with standard rat chow (0.25% Na, 14.7% proteins), and the TTA group was fed 1g TTA dissolved in 1 liter of acetone to 2.7 kg pellet). We have shown previously that TTA and acetone did not interfere with the food intake (5, 16).

**Collection of urine.** Urine samples were collected from unanesthetized rats 6 wk after clipping and the start of TTA treatment. The urine was collected on ice for 24 h using metabolic cages. The urine volume was recorded as milliliter per hour, and 1 ml of each sample was filtered and frozen at −80°C until analyzed.

**Collection of renal cortex.** The kidneys were collected from anesthetized rats and immediately frozen in liquid nitrogen. When homogenized, the kidneys were kept for 30 s at room temperature, and the cortex was isolated, weighted, and homogenized in lysis buffer (5 mM HCl, 10 mM KH2PO4, 1.5 mM MgCl2, 10 mM KCl, 0.1% Triton X-100, and EDTA-free proteinase inhibitor mixture; Roche). The homogenized samples were centrifuged at 10,000 g for 15 min at 4°C, and the supernatant was kept at −80°C until analyzed.

**Measurements of COX1 and COX2 activity in renal cortex.** COX1 and COX2 activity was measured using a kit for the peroxidase activity of COX (no. 760151; Cayman Chemical). The peroxidase activity was assayed colorimetrically by monitoring the appearance of oxidized N,N,N',N'-tetramethyl-p-phenylenediamine (TMPD), a stable metabolite of PGH2, in the presence of arachidonic acid. When assayed, the samples were treated with SC-560 (COX1 selective inhibitor) and DuP-697 (COX2 selective inhibitor). Inactive samples, obtained by placing these in boiled water 5 min, were assayed as background for each sample. Arachidonic acid was added, and the oxidized PGH2 was quantified colorimetrically at 590 nm. The reaction rate was determined using the TMPD extinction coefficient (0.00826 μM⁻¹). One unit was defined as the amount of enzyme that will cause the oxidation of 1.0 nmol of TMPD/min at 25°C. The total COX activity for each sample was calculated in nmol·min⁻¹·g⁻¹ (U/ml). The COX1 or COX2 activity in each sample was calculated as a percentage from total COX activity.

**Western blot for COX1 and COX2 from kidney cortex.** Homogenates of kidney cortex (1 g tissue/5 ml buffer) were prepared in 25 mM Tris·HCl, pH 7.4, 0.5 mM EDTA, 0.5 mM EGTA, and 1 mM phenylmethylsulfonyl fluoride with proteinase inhibitor mixture (Roche). The homogenate was centrifuged, and the resulting supernatant was collected as the cytosolic fraction. The COX1 and COX2 expressions were assessed by standard SDS-PAGE Western blotting techniques. The proteins were separated by electrophoresis on a 10% SDS-PAGE with Tris glycerol buffer (10% SDS, 24% glycerol, 1.5% Triton X-100, and 192 mM glycine) for 1 h at 150 volts. The transfer was made for 1 h at 100 volts on a polyvinylidene difluoride transfer membrane (Amersham). The membrane was blocked by skimmed milk in Tris-buffered salt solution (TBS: 19 mM Tris and 0.5 mM NaCl) for 1 h at room temperature and washed with TBS-Tween (TBS with 0.05% Tween 20). The membrane was then incubated with mouse monoclonal anti-COX1 antibody (11 SC-19998) or mouse monoclonal anti-COX2 antibody (29 SC-19999; Santa Cruz Biotechnology) for 1 h at room temperature. After being washed (3 × 10 min with TBS-Tween at room temperature), the membrane was incubated with secondary antibody (SC-2005; Santa Cruz Biotechnology).

The protein marker was a dual-color one from Bio-Rad (catalog no. 161.1374). Enhanced chemiluminescence detection was used (ECL from Amersham). The membranes were developed by a Bio-Rad Laboratory utility. Actin was used as loading reference, and Western blot detection was done by the same protocol as for COX1 and COX2. Antibodies used were as follows: mouse monoclonal (4374 SC-70318) and secondary antibody (SC-2005; Santa Cruz Biotechnology) antibody. Densitometric analyses of the blots were done by Image J software, and the ratio COX1/actin and COX2/actin was calculated.

**Measurement of PGE2 in kidney cortex and urine.** The PGE2 was measured by an enzymatic immunoassay kit from Cayman Chemical (catalog no. 514531.1). The assay was based on the competition between the stable metabolite of PGE2 (PGEM) and PGEM-acetylcholinesterase conjugate as tracer against a limited number of PGEM-specific rabbit antiserum-binding sites. The unbound reagents were removed by wash, and the substrate for the acetylcholinesterase (Elmans reagent) was added. The absorbance was recorded with a 412-nm filter.

**Real-time quantitative RT-PCR for PPARs.** Total RNA was purified from frozen kidneys using the RNeasy Midi Kit (Qiagen, Hilden, Germany). PPARα, PPARδ, and PPARγ are "Assay-on-Demand genes" designed by Applied Biosystems, with the following assay ID numbers: Rn00561953_m1 (PPARα), Rn00567507_m1 (PPARδ), and Rn00440945_m1 (PPARγ). Real-time PCR was carried out in triplicate for each sample on an ABI 7900 sequence detection system (Applied Biosystems). A dilution curve from one cDNA source using antibodies 1:2, 1:4, 1:8, and a no-template control was run for each gene. The gene expression was determined by relative quantification using the standard curve method. For each sample, results were normalized to 18S rRNA (RT-CKFT-18S; MedProbe) by comparing changes in threshold cycles.

**Hemodynamic study.** The left kidney from control rats and the left nonclipped kidney from 2KIC and TTA-treated rats were used for measurements of RBF in response to ANG II injections in the renal artery 6 wk after clipping. The ANG II concentration (2.5 ng) used did not change the systemic BP recorded as mean arterial pressure (MAP).
The experiments have been described previously (8). In short, anesthesia was induced by an intraperitoneal injection of pentobarbital sodium (65 mg/kg body wt), and the rats were placed on a servo-controlled heating table to keep the body temperature at 38.0 ± 0.5°C. Tracheotomy was performed to facilitate free breathing, and the animals received oxygen during the acute experiment.

The right femoral artery was cannulated with a PE-25 catheter to monitor arterial pressure using a Statham P 23 XL pressure transducer. Another PE-25 catheter was introduced in the right femoral vein for the infusion of albumin, injection of indomethacin, and supplementary doses of pentobarbital sodium. BSA (4.7 g/dl) was dissolved in isotonic saline containing heparin (0.15 ml/10 ml saline). The BSA solution was infused initially at a rate of 50 µl/min to replace losses associated with surgery (1.25 ml 100 g−1·kg body wt−1) and then at 10 µl/min during the experiment.

The abdominal aorta and left kidney were exposed through a midline incision. For injection of ANG II, a PE-10 catheter was introduced in the internal iliac artery and then advanced until its tip was positioned ~2–3 mm inside the left renal artery without affecting the RBF. The left renal artery was localized and dissected free. The adjacent fat tissue was carefully removed for proper acoustical coupling. An ultrasound V Transonic System flow probe with an internal diameter of 0.8 mm was placed on the renal artery. The Dane-Gel E2 (Rhode Products) aqueous transmission was used as an acoustical couplant. The flow probe was interfaced to the data acquisition system using a Transonic T 206 electromagnetic flowmeter and a Gould TA 5000 recorder installed on a PC compatible computer with a 12-bit analog-to-digital converter. A Gould TA 5000 Hewlett Packard carrier amplifier was used for the pressure transducer sensor interface. The outputs of the transducer monitoring arterial pressure and RBF were sampled at a rate of 1 sample/s. Each recording started 15 s before injection of vasoconstrictor (ANG II) in left renal artery. RBF values were measured in milliliters per minute and expressed as a percent of baseline values.

After completion of the surgical preparation, the animals were allowed to stabilize for 1 h before starting the records of MAP and RBF response to ANG II injections in left renal artery. Initially, the renal vascular response to 2.5 ng ANG II was recorded as a basic line vascular response. Thereafter, indomethacin (1 ml/kg body wt) was infused through the right venous femoral catheter. Indomethacin was prepared by diluting 5 mg indomethacin in 1 ml distilled water, buffered by 5 mg sodium carbonate. Injections of ANG II were performed 20 min after indomethacin treatment, and the RBF response to 2.5 ng ANG II was recorded as a vascular response. At the end of experiments, the rats were killed by an extra dose of pentobarbital sodium. The kidneys were removed and weighed.

Infusion of a COX2 inhibitor. A separate group of 12 animals was prepared for 2K1C as described above and subcutaneously infused with the specific COX2 inhibitor parecoxib (5 mg·kg−1·24 h−1; Pfizer) using osmotic minipumps (2ML4; Alzet) for 4 wk after surgery. BP was investigated at the end of treatment using the tail-cuff method described above.

Chemicals. ANG II, BSA, and indomethacin were acquired from Sigma-Aldrich. Heparin (100 IE/ml) was bought from Leo-Pharma.

Statistical methods. Data are reported as means ± SE. Statistical analyses were done using SPSS for Windows 2003. Differences between groups were assessed by one-way ANOVA, followed by post hoc Scheffé correction. For RBF measurements before and after indomethacin, a paired Student’s t-test was used to assess the differences within the same group. P < 0.05 was considered statistically significant.

RESULTS

Systolic BP. The BP was not significantly different between the groups before clipping, and the BP in the control group was unchanged after 6 wk (108 ± 3 mmHg). In the 2K1C hypertensive group, the BP was increased significantly to 183 ± 4 mmHg 6 wk after clipping compared with 110 ± 2 mmHg before clipping (P < 0.001) and with 108 ± 3 mmHg in controls (P < 0.001). In the 2K1C TTA-treated group, BP was significantly lower 6 wk after clipping compared with 2K1C (128 ± 3 mmHg, P < 0.001), but the BP remained higher than before clipping (P < 0.001) (Fig. 1).

COX1 activity in renal cortex. COX1 activity was calculated as the percentage of the total COX activity. In the kidney cortex from the control group, the COX1 activity was 80 ± 3%. In the clipped kidney from 2K1C rats, the COX1 activity was reduced to 61 ± 4% (P = 0.004 compared with control), whereas in the TTA-treated 2K1C group, COX1 activity was similar to control values (83 ± 3%) and significantly higher than the 2K1C group (P = 0.001) (Fig. 2A). In the nonclipped kidney of the 2K1C group, the COX1 activity was 66 ± 3% (P = 0.009 compared with control). After TTA treatment in the 2K1C group, the COX1 activity in this kidney was similar to control (76 ± 2%) and higher compared with the 2K1C group (P = 0.02) (Fig. 2C).

COX2 activity in renal cortex. The COX2 activity was calculated as the percentage of the total COX activity. The COX2 activity in the renal cortex from the control was 16 ± 3%. In the clipped kidney of the 2K1C group, COX2 activity was nearly two times higher (38 ± 2%) compared with controls (P < 0.001), and after TTA treatment, the COX2 activity in this kidney was similar to controls (18 ± 2%), but lower when compared with the 2K1C group (P < 0.001) (Fig. 3C). In the nonclipped kidney of 2K1C, the COX2 activity was lower than in the clipped kidney, but still significantly higher than controls (26 ± 2%, P = 0.02). In the TTA-treated 2K1C group, COX2 activity in the nonclipped kidney was 22 ± 2%, similar to the control value, but lower compared with the 2K1C group (P = 0.04) (Fig. 3C).

Protein expression for COX1. COX1 was detected at 70 kDa. There was no difference in COX1 protein expression...
between the groups. The ratio COX1/actin was 1.6 ± 0.08 in control group, while in 2K1C group was 1.3 ± 0.11 in the clipped kidney and 1.2 ± 0.09 in the nonclipped kidney. After TTA treatment, the ratio COX1/actin was 1.4 ± 0.12 in the clipped kidney and 1.6 ± 0.09 in the nonclipped kidney compared with 2K1C. The TTA-treated group is not different from the control group.

**Protein expression for COX2.** COX2 was detected at 74 kDa. The ratio COX2/actin expression in the control group was 1.1 ± 0.04. The COX2/actin expression ratio was higher in both kidneys of the 2K1C group. The ratio COX2/actin expression was higher than in controls in both the clipped kidney (1.6 ± 0.12, \(P = 0.006\)) and nonclipped kidney (1.3 ± 0.04, \(P = 0.009\)) compared with controls (Fig. 3A). In the TTA-treated 2K1C group, the ratio COX2/actin expression was similar to controls (1.2 ± 0.15 in the clipped kidney and 1.1 ± 0.09 in the nonclipped kidney, \(P \leq 0.02\) compared with 2K1C) (Fig. 3A). Representative blots for COX2 and actin are shown in Fig. 3B.

**PGE2 urine and renal cortex concentration.** The PGE2 level in the kidney cortex of the control group was 171 ± 10 pg/g. In the clipped kidney of the 2K1C group, the PGE2 level was 288 ± 26 pg/g (\(P = 0.001\) compared with controls) and 198 ± 4 pg/g in the nonclipped kidney (\(P = 0.03\) compared with controls). In the 2K1C TTA-treated group, the PGE2 levels were lower both in the clipped (173 ± 19 pg/g, \(P < 0.001\)) and nonclipped kidney compared with 2K1C.

Fig. 2. Cyclooxygenase 1 (COX1) protein expression (A and B) and COX1 activity (C) in the kidney cortex in control, 2K1C, and 2K1C TTA groups (n = 6 in each group). A: *\(P = 0.03\), increased COX1 protein expression in 2K1C TTA-treated group compared with 2K1C. B: representative Western blot for COX1 and actin detection. C: *\(P < 0.01\), reduction of COX1 activity in the clipped and nonclipped kidney of 2K1C group compared with control. **\(P \leq 0.02\), enhancement of COX1 activity after TTA treatment in both clipped and nonclipped kidney compared with 2K1C. The TTA-treated group is not different from the control group.

Fig. 3. COX2 protein expression (A and B) and COX2 activity (C) in the kidney cortex control, 2K1C, and 2K1C TTA groups (n = 6 in each group). A: *\(P < 0.001\), upregulation of COX2 protein expression in both clipped and nonclipped kidney compared with control. **\(P \leq 0.02\), reduction of COX2 protein expression after TTA treatment in both kidneys compared with 2K1C. B: representative Western blot for COX2 and actin detection. C: *\(P < 0.02\), reduction of COX2 activity in the clipped and nonclipped kidney of 2K1C group, both compared with control. **\(P < 0.04\), enhancement of COX1 activity after TTA treatment in both clipped and nonclipped kidney compared with 2K1C. The TTA-treated group is not different from the control group.
compared with 2K1C) and nonclipped kidney (170 ± 11 pg/g, P < 0.001 compared with 2K1C) kidney, but the levels in 2K1C TTA kidneys were similar to controls in the clipped kidney (Fig. 4).

The PGE2 concentration in urine from the control group was 2.4 ± 0.05 pg/ml. The PGE2 concentration was 3.5 ± 0.1 pg/ml in the urine from the 2K1C hypertensive rats (P < 0.001, compared with controls). After TTA treatment, PGE2 concentration was similar to control values (2.4 ± 0.05 pg/ml) and significantly lower when compared with 2K1C (P < 0.001) (Fig. 5).

Real-time quantitative RT-PCR for PPARs. The mRNA levels of all PPARs were measured in both the clipped and the nonclipped kidney. The effect of TTA treatment increased the mRNA level of PPARδ in the nonclipped kidney. There was a tendency for a lowering effect of TTA on PPARα and PPARδ in clipped kidneys (Fig. 6).

Infusion of a COX2 inhibitor. Four weeks of infusion of the COX 2 inhibitor did not change BP, which was 174 ± 5 mmHg after infusion compared with 176 ± 4 mmHg in the untreated 2K1C hypertensive animals.

RBF response to ANG II after indomethacin treatment. Baseline RBF before indomethacin treatment was 6.3 ± 0.4 ml·min⁻¹·g⁻¹ in the left kidney of controls, 6.4 ± 0.2 ml·min⁻¹·g⁻¹ in the nonclipped kidney of the 2K1C group, and 6.5 ± 0.2 ml·min⁻¹·g⁻¹ in the nonclipped kidney of the TTA-treated group. Inhibition of the COX system by indomethacin did not change baseline RBF or BP in any of the groups.

ANG II injected in the renal artery decreased RBF in all groups. In the control group, the RBF decrease was accentuated from 21 ± 3% before to 44 ± 6% after indomethacin (P = 0.002). In the nonclipped kidney of 2K1C, the RBF decrease was also strengthened from 10 ± 2% before to 19 ± 2% after indomethacin treatment (P = 0.003) (Fig. 7). TTA treatment blunted this effect. The RBF response to ANG II was 20 ± 3% before and 23 ± 4% after indomethacin (P = 0.1) in the TTA-treated 2K1C animals (Fig. 7).

DISCUSSION

The main new information in the present study is that TTA, a modified fatty acid, attenuated COX2 activity and protein expression in both clipped and nonclipped kidneys from rats with 2K1C hypertension. This may affect renal vascular reactivity and have effects on the regulation of renal hemodynamics. However, it does not appear to be the major mechanism for TTA’s effect on BP, since neither acute general COX inhibition nor chronic specific COX2 inhibition was able to lower BP.

As shown previously (19, 24, 27), we found that COX2 activity was enhanced in the clipped kidney and, to some extent, also in the nonclipped kidney of untreated rats (33). In contrast, COX1 expression was unchanged in both kidneys of 2K1C, and the activity compared with total COX activity was reduced in proportion to the increase in COX2 activity. There may be a component of direct COX1 downregulation in our model, since there is a tendency toward lower values in both kidneys that corroborates results from other investigators (50). Interestingly, the TTA treatment normalized the activity of both COX1 and COX2 in both kidneys, again indicating that TTA affects a common controller upstream in the 2K1C model. The results for COX activity were borne out in that the renal content of PGE2 was nearly doubled in the clipped kidney compared with the nonclipped kidney, and PGE2 urinary output was increased in untreated 2K1C when compared with controls. Further TTA treatment normalized both the cortex level and the urinary output of PGE2, showing that the effect of TTA on COX2 expression and activity is not buffered at some other point in the system.

As previously shown, the RBF response to ANG II injection in the renal artery of the nonclipped kidney was increased by indomethacin pretreatment both in controls (15) and, as we have shown in isolated vessels (22), in 2K1C animals. However, in TTA-treated 2K1C animals, indomethacin did not alter the magnitude of the RBF response. This further supports that TTA downregulates COX2 activity and PG production, which has important effects on the renal vascular reactivity.

In the present study, we have also confirmed that TTA partially prevents the increase of BP in 2K1C rats, as we have shown previously (5, 16). The fact that neither indomethacin nor parecoxib has any effect on BP in 2K1C rats further supports that the TTAs effect on BP is at an upstream site,
potentially renin expression and release. This is consistent with our previous results showing that TTA has no effect on BP in spontaneously hypertensive rats that have low plasma renin concentration, as well as with earlier studies showing that renin gene expression and COX2 expression have a parallel expression in the rat cortex in renal hypertension (21). However, COX2 inhibition has been found to lower BP in renovascular hypertension in 2K1C rats (33) and in rats with aortic coarctation (56), which is contrary to our results, while others have found no effect of COX2 inhibition on BP (41). These differences must for the time being be attributed to differences in models and strains but may also indicate COX2-independent effects of some of the inhibitors used. This clearly warrants further study.

TTA exerts various effects via PPAR-dependent and -independent pathways (3, 14). The blood pressure-lowering effect of other PPAR-activating drugs, such as the PPARγ ligand thiazolidinedione, as reviewed previously (9, 13, 42), is linked to renin expression, as we and others have shown previously (16, 44). However, the precise mechanism is not clear, since PPARγ activation in human renin-producing cells in vitro has been shown to increase renin transcription (51, 52). The effect of PPAR activation on COX2 activity is consistent with previous findings in the kidney (46) and reinforces that TTA may work through a PPAR-dependent pathway in affecting BP in the 2K1C rat. In the present study, we observed an increase of PPARγ mRNA in the nonclipped kidney, and there was a tendency toward lower expression of both PPARα and PPAR-δ in the clipped kidney. No effect was seen on PPARγ. These results suggest that PPARs are indeed expressed in the kidney and that PPARγ, which is most closely connected to BP regulation, is not significantly changed by TTA treatment.

The COX enzymes catalyze prostaglandin synthesis from free arachidonic acid (48, 53). There are three COX isoenzymes that are encoded by different genes, COX1, COX2 and COX3 (11), which transform arachidonic acid in a two-step reaction into PGG2/PGH2, which is further metabolized to a variety of prostanoids by specific enzymes (47, 53). COX2 is the inducible form connected with different pathological situations that activates inflammatory pathways (19), although it is well known that COX2 is also constitutionally expressed in the thicken ascending limb of the loop of Henle, medullary interstitial cells, and macula densa cells (19, 48). COX2 is upregulated in both clipped and nonclipped kidney of 2K1C (24), as we found 6 wk after clipping in the present study. We have previously shown that COX2 inhibition does not affect the reactivity of isolated afferent arterioles to ANG II (22). Thus it is reasonable to suggest that the effects we describe on RBF regulation are related to changes of COX2 activity in the macula densa with corresponding effects on renin regulation (38) and renal autoregulation (12, 18, 37).

An alternative explanation is that the increased PG production is related to renal hypertensive damage and inflammation, which is then decreased when TTA lowers BP. It has been reported that PGE2 production in inflammation is COX2 dependent (48), and in a recent study (6), we have shown that the inflammatory marker nuclear factor-κB (NF-κB) is activated in 2K1C hypertension in both kidneys. This is of interest since it...
has been shown that NF-κB-dependent activation of interleukin (IL)-1β and tumor necrosis factor (TNF)-α, leads to COX2 activation (54). There is also evidence regarding IL-1β- and TNF-α-dependent COX2 stimulation (39), and we have found upregulation of TNF-α and IL-1β concentrations both in plasma and in kidney tissue in 2K1C rats (6). Another mechanism that could play a role in the changes in PG concentrations could be the fact that TTA has been shown to upregulate dihomo-γ-linolenic acid (DGLA) and downregulate eicosapentaenoic acid (EPA) in serum of 2K1C rats, whereas the serum level of arachidonic acid (AA) was not affected (16). DGLA, EPA and AA are competitive substrates for COX2 (49), which could have effects beyond the simple effects on expression.

Perspectives and Significance

From the presented results, we conclude that TTA downregulates COX2 synthesis in both clipped and nonclipped kidney in a 2K1C hypertensive rat model, which has potentially important effects on the regulation of RBF. The inability of indomethacin and parecoxib to reduce BP indicates that TTA’s effect on COX2 does not play a role in the effect on BP. On the basis of findings that TTA prevents high BP and reduces the activity of the renin-angiotensin system, NF-κB, and cytokines (6), TTA could be a promising drug for patients with renovascular hypertension.

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