Prostaglandins that increase renin production in response to ACE inhibition are not derived from cyclooxygenase-1

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Cheng, Hui-Fang, Sue-Wan Wang, Ming-Zhi Zhang, James A. McKanna, Richard Breyer, and Raymond C. Harris. Prostaglandins that increase renin production in response to ACE inhibition are not derived from cyclooxygenase-1. Am J Physiol Regul Integr Comp Physiol 283: R638–R646, 2002;—It is well known that nonselective, nonsteroidal anti-inflammatory drugs inhibit renal renin production. Our previous studies indicated that angiotensin-converting enzyme inhibitor (ACEI)-mediated renin increases were absent in rats treated with a cyclooxygenase (COX)-2-selective inhibitor and in COX-2−/− mice. The current study examined further whether COX-1 is also involved in mediating ACEI-induced renin production. Because renin increases are mediated by cAMP, we also examined whether increased renin is mediated by the prostaglandin E2 receptor EP2 subtype, which is coupled to Gα and increases cAMP. Therefore, we investigated if genetic deletion of COX-1 or EP2 prevents increased ACEI-induced renin expression. Age- and gender-matched wild-type (+/+ ) and homozygous null mice (−/−) were administered captopril for 7 days, and plasma and renal renin levels and renal renin mRNA expression were measured. There were no significant differences in the basal level of renal renin activity from plasma or renal tissue in COX-1+/+ and −/− mice. Captopril administration increased renin equally [plasma renin activity (PRA): +/+ 9.3 ± 2.2 vs. 50.1 ± 10.9; −/− 13.7 ± 1.5 vs. 43.9 ± 6.6 ng ANG I·ml−1·h−1; renal renin concentration: +/+ 11.8 ± 1.7 vs. 35.3 ± 3.9; −/− 13.0 ± 3.0 vs. 27.8 ± 5.7 ng ANG I·mg−1·protein−1·h−1; n = 6; P < 0.05 with or without captopril]. ACEI also increased renin mRNA expression (+/+ 2.4 ± 0.2; −/− 2.1 ± 0.2 fold control; n = 6–10; P < 0.05). Captopril led to similar increases in EP2−/− compared with +/+. The COX-2 inhibitor SC-58236 blocked ACEI-induced elevation in renal renin concentration in EP2 null mice (+/+ 24.7 ± 1.7 vs. 9.8 ± 0.4; −/− 21.1 ± 3.2 vs. 9.3 ± 0.4 ng ANG I·mg−1·protein−1·h−1; n = 5) as well as in COX-1−/− mice (SC-58236-treated PRA: +/+ 7.3 ± 0.6; −/− 8.0 ± 0.9 ng ANG I·ml−1·h−1; renal renin: +/+ 9.1 ± 0.9; −/− 9.6 ± 0.5 ng ANG I·mg−1·protein−1·h−1; n = 6–7; P < 0.05 compared with no treatment). Immunohistochemical analysis of renin expression confirmed the above results. This study provides definitive evidence that metabolites of COX-2 rather than COX-1 mediate ACEI-induced renin increases. The persistent response in EP2 nulls suggests involvement of prostaglandin E2 receptor subtype 4 and/or prostacyclin receptor (IP).

angiotensin-converting enzyme inhibitor; cyclooxygenase-2 −/−; prostaglandin E2 receptor subtype 2

IN THE KIDNEY, prostaglandins (PGs) are important mediators of vascular tone and salt and water homeostasis and are involved in the mediation and/or modulation of hormonal action (37). PGs arise from enzymatic metabolism of free arachidonic acid by cyclooxygenase (COX), the enzyme responsible for the initial rate-limiting metabolism of arachidonic acid to PGG2 and subsequently to PGH2, followed by further metabolism by specific synthetases. Two isoforms of COX have been identified, COX-1 and COX-2. COX-1 is expressed constitutively in the kidney and is localized predominantly in mesangial cells, arteriolar endothelial cells, parietal epithelial cells of Bowman’s capsule, and cortical and medullary collecting ducts (21, 44). It has been determined that COX-1 is not primarily responsible for the increased prostanoid production in inflammatory states nor is it glucocorticoid sensitive (35). In the kidney, COX-2, the “inducible” COX, is localized in macula densa cells and surrounding cortical thick ascending limb (cTAL) cells and in a subset of medullary interstitial cells near the papillary tip in normal adult kidney (21, 44). Macula densa/cTAL expression increases in high renin states, such as dietary salt depletion (21, 25), angiotensin-converting enzyme (ACE) inhibitor treatment (11, 50), diuretic administration (34), or experimental renovascular hypertension (22, 48).

Prostanoids act locally via specific transmembrane G protein-coupled receptors. In the kidney cortex, PGE2 is the major PG produced (7). Four EP receptor subtypes have been cloned (3, 31). EP1 and EP3 receptor mRNA expression are detected in the collecting duct and thick limb, respectively (17, 45), whereas the EP4 receptor is widely expressed compared with other EP receptors, with the highest concentrations in the glomerulus (6, 8). The human EP2 receptor subtype protein was detectable only in the media of arteries and...
arterioles. Although the EP\textsubscript{2} receptor exhibits a low level expression in the kidney, recent studies demonstrated that the EP\textsubscript{2} receptor may be a mediator of arterial dilatation and salt-sensitive hypertension (30).

In previous studies, we determined that administration of a selective COX-2 inhibitor prevented increases in ACE inhibitor (ACEI)-mediated renin release (12). We also found that ACEI failed to increase renin in mice with genetic deletion of COX-2. Others also determined that COX-2 inhibition blunted renin expression in response to dietary sodium deprivation (19, 20, 28, 51). However, this issue remains controversial because other groups do not report changes in renin expression in response to COX-2 inhibitors (23, 29, 34, 40). To further investigate the role of COX metabolites in regulation of renin expression and release, the current studies used mice with genetic deletion of the COX-1 gene to investigate whether COX-1 is involved in mediation of ACEI-induced renin release. We also studied the potential role of the EP\textsubscript{2} receptor in mediation of renin release.

**MATERIALS AND METHODS**

*Animals and genotyping.* Mice with genetic deletion of the COX-1 gene (COX-1\textsuperscript{−/−}) and maintained on a mixed B6/129 background were a generous gift from Robert Langenbach (33). Mice were genotyped by PCR. The employed primer pairs were IMR013 (CCTGGGTGAGAGGCTATTC) and IMR014 (AGGTGAGTGCAGGGAGATC), and the specific mouse COX-1 primers were (GAGAGGAGGATGCTGCTAGTGTG) and (AGGTGAGTGCAGGGAGATC). PCR was performed for 35 cycles at 95°C for 30 s, 62°C for 45 s, and 72°C for 90 s, followed by a 10-min extension at 72°C. Because the neocassette replaced a portion of the 5’ exon 11 and surrounding introns, the neoprimers 0IMR13 and 0IMR14 amplified a 280-bp product in homozygous or heterozygous mice, whereas the specific COX-1 primers generated a 1,016-bp product from the wild-type and heterozygous mice (Fig. 1A). The EP\textsubscript{2} receptor-deleted mice were maintained on a BALB/c background as previously described (30). Mice were genotyped by Southern hybridization with a specific 3’ probe. For Southern analysis, DNA samples were digested with XbaI, electrophoresed in agarose gels, and transferred to nylon transfer membranes. The blots were hybridized as described (12). Wild-type (+/+), heterozygous (+/−), and homozygous (−/−) knockout offspring are indicated in Fig. 1B.

Age-matched male adult (6–8 wk) COX-1 +/+ , +/−, and −/− and EP\textsubscript{2} +/+ and −/− mice were divided into three groups: 1) control with vehicle only, 2) administration of 400 mg/l captopril in drinking water, and 3) captopril combined with the COX-2-selective inhibitor SC-58236 (10 mg/l) in drinking water for 7 days (11), calculated to provide a dosage of 3–5 mg kg\textsuperscript{−1} day\textsuperscript{−1}. SC-58236 was dissolved in vehicle (final concentration: 0.01% Tween 20 and 0.2% PGE-200 (29)), and all animals received equal amounts of vehicle in their drinking water.

*RNA extraction and Northern blotting.* Kidney RNA was extracted by the acid guanidium thiocyanate-pheno1 chloroform method (11). RNA samples were electrophoresed in denatured agarose gel, transferred to nitrocellulose membranes, and hybridized with a 1.4-kb \textsuperscript{32}P-labeled cDNA fragment of rat renin (48). The membranes were then stripped and rehybridized with glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

*Immunohistochemistry.* Under deep anesthesia with Nembutal (70 mg/kg ip), mice were perfused in situ with saline containing 0.02% sodium nitrite and heparin (10 U/ml) and fixed by perfusion for renin immunohistochemistry with 3.7% formaldehyde, 1.4% lysine, 0.01 M sodium metaperiodate, 0.04 M sodium phosphate, and 1% acetic acid. The kidneys were then dehydrated with a graded series of ethanols and embedded in paraffin. Sections (4-μm thick) were mounted on glass slides and immunostained with polyclonal rabbit anti-renin antiserum (1:6,000 dilution), a generous gift from Prof. T. Inagami, Vanderbilt University. Vectastain ABC-Elite (Vector, Burlingame, CA) was used to localize the primary antibody with a chromogen of oxidized diaminobenzidine, followed by a light counterstain with toluidine blue.

*Renin activity.* Animals were killed by inhalation anesthe-sia with isoflurane (Baxter Pharmaceutical Products, Deer-
field, IL). Blood was collected in EDTA (1 mg/ml blood) on ice at the time of death. The plasma was separated and frozen at −20°C until assay. For measurement of renal tissue renin concentration, the kidneys were homogenized in 0.1 M Tris-HCl, pH 7.4, containing (in mM) 3.4 8-hydroxyquinoline sulfate, 0.25 EDTA, 0.1 phenylmethylsulfonyl fluoride, 1.6 dimercaprol, 5 sodium tetrathionate, and 0.1% Triton X-100. The concentration of protein was determined with a bicinchoninic acid protein assay kit (Pierce, Rockford, IL). After centrifugation of the homogenates, the supernatant was incubated for 1 h with excess exogenous renin substrate (plasma obtained from mice nephrectomized 48 h before collection). Renin was analyzed by radioimmunoassay with an 125I-ANG I kit (New England Nuclear).

Statistical analysis. All values are presented as means ± SE. ANOVA and Bonferroni t-tests were used for statistical analysis, and differences were considered significant when P < 0.05.

RESULTS

Plasma renin activity (PRA) was measured at the time of death (±7 days of captopril treatment). Captopril significantly increased PRA in COX-1+/+ (9.3 ± 2.2 vs. 50.1 ± 10.9 ng ANG I·ml⁻¹·h⁻¹),+/− (6.6 ± 1.4 to 37.2 ± 2.1 ng ANG I·ml⁻¹·h⁻¹), and −/− (13.7 ± 1.5 vs. 43.9 ± 6.6 ng ANG I·ml⁻¹·h⁻¹) mice (n = 6; P < 0.05 for all groups comparing with and without captopril administration; Fig. 2A). The COX-2-specific inhibitor SC-58236 did not alter basal cortical COX-2 mRNA expression in control wild-type mice (Fig. 2B). However, addition of SC-58236 to the ACEI-treated animals led to comparable inhibition of the increases in PRA in all three groups (+/+: 7.3 ± 0.6, +/−: 7.2 ± 1.2, −/−: 8.0 ± 0.9 ng ANG I·ml⁻¹·h⁻¹; Fig. 2A).

Renal renin mRNA was normalized with GAPDH and expressed as fold of control (+/− mice without treatment). In studies investigating COX-1 knockouts, there were no significant differences in the basal level of renin mRNA among the three genotypes (1.00 ± 0.03 fold of control in +/− and 1.00 ± 0.06 fold of control in −/− mice, respectively; n = 6; not significant). Captopril treatment increased renin mRNA significantly in all of the three genotypes (2.4 ± 0.2 fold of control in +/+, 2.4 ± 0.2 fold of control in +/−, and 2.1 ± 0.2 fold of control in −/−; n = 9; P < 0.01), and no significant differences were found among the groups (Fig. 2C).

Fig. 2. COX-1 knockout mice. A: plasma renin activity (PRA) in response to captopril treatment. Animals were administered captopril (400 mg/l) in drinking water for 7 days (n = 6; **P < 0.01). B: similar expression of COX-2 mRNA in renal cortex of wild-type B6/129 mice with or without 7-day treatment with the COX-2 inhibitor SC-58236. Lanes 1 and 3: control; lanes 2 and 4: SC-58236. C: effect of the captopril on renal renin mRNA expression. Animals were treated as described in A. Renin mRNA was detected by Northern blotting and normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA expression. Inset: representative experiment. Lanes 1, 4, and 7: without captopril treatment; lanes 2, 5, and 8: with captopril treatment; lanes 3, 6, and 9: with combined captopril and SC-58236 treatment; +/+; Lanes 1-3; +/−: lanes 4-6; −/−: lanes 7-9 (n = 6; **P < 0.01). D: renal renin concentration (RRC) in response to captopril treatment. Animals were treated as described in A. Renal renin activity was determined as described in MATERIALS AND METHODS (n = 6; **P < 0.01).
Fig. 3. Immunoreactive renin expression in the kidney of COX-1−/− mice. A, B, C: +/+ without (A), with (B) captopril, or with both captopril and SC-58236 treatment (C). D, E, F: +/− without (D), with (E) captopril, or with the combined captopril and SC-58236 treatment (F). G, H, I: −/− without (G), with (H) captopril, or with both (I). Arrows in B, E, and H indicate afferent arteriole proximal to juxtaglomerular region, with evidence of renin upregulation in all 3 genotypes. SC-58236 reversed the elevated renin expression (image width = 700 μm).
SC-58236 prevented increases in renal renin mRNA expression in all three groups (1.2 ± 0.1 fold of control in +/+; 1.2 ± 0.2 fold of control in +/-, and 1.2 ± 0.2 fold of control in −/−; n = 5; P < 0.01; Fig. 2C).

Renal renin concentration (RRC) was also significantly elevated by the administration of captopril (+/+: 11.8 ± 1.7 to 35.3 ± 3.9, +/-: 7.6 ± 0.9 to 27.6 ± 3.2, and −/−: 13.0 ± 3.1 vs. 27.8 ± 2.7 ng ANG I·mg protein⁻¹·h⁻¹; n = 6; P < 0.05 with or without captopril). There were no significant differences in either PRA or RRC among the three phenotypes (Fig. 2C).

SC-58236 prevented increases in RRC (+/+: 9.1 ± 0.9, +/-: 8.7 ± 0.6, and −/−: 9.6 ± 0.5 ng ANG I·mg protein⁻¹·h⁻¹; Fig. 2C).

Immunoreactive (ir) renin was localized to the juxtaglomerular (JG) cells in all three groups of mice (Fig. 3). In agreement with the above results, captopril administration increased ir-renin expression (Fig. 3, B, E, and H) and SC-58236 equally blunted ACEI-induced increases in renin expression in wild-type heterozygotes and COX-1 knockout mice (Fig. 3, C, F, and I).

Determination of renin expression was also performed in EP 2 +/- and −/− mice. Captopril led to similar increases of PRA in EP 2 +/- and EP 2 −/− (14.8 ± 1.4 to 39.0 ± 6.7 ng ANG I·ml⁻¹·h⁻¹; n = 5; P < 0.05 vs. 13.9 ± 2.9 to 38.3 ± 7.5 ng ANG I·ml⁻¹·h⁻¹; n = 5; P < 0.05). The COX-2 inhibitor SC-58236 blocked ACEI-induced elevations in PRA in EP 2 −/− mice (+/+: 13.1 ± 2.9 ng ANG I·ml⁻¹·h⁻¹; −/−: 13.5 ± 2.5 ng ANG I·ml⁻¹·h⁻¹; n = 5; P < 0.05; Fig. 4A). Renal renin mRNA levels were also upregulated to the same extent in both groups by the administration of captopril (+/+: 2.6 ± 0.3 fold control; −/−: 2.7 ± 0.6 fold control; n = 4; P < 0.05 with or without captopril), and this increase was prevented by the addition of SC-58236 (+/+: 1.1 ± 0.1 fold control; −/−: 1.3 ± 0.3 fold control; n = 4; P < 0.05; Fig. 4B). Figure 4C indicates the results of RRC (basal level/captopril/additional SC-58236 in +/-: 11.0 ± 0.8/24.7 ± 1.7/9.8 ± 0.4 ng ANG I·mg protein⁻¹·h⁻¹; −/−: 9.8 ± 0.7/21.1 ± 3.2/9.3 ± 0.4 ng ANG I·mg protein⁻¹·h⁻¹; n = 5; P < 0.05, captopril group compared with control or additional SC-58236-treated group). Correspondingly, immunohistochemistry indicated that captopril administration led to comparable increases in renin expression in JG cells of wild-type and EP-2 knockout mice (Fig. 5, B and E), and SC-58236 blunted this increase in both groups (Fig. 5, C and F).

Fig. 4. EP 2 receptor −/− mice. A: PRA in response to captopril treatment. Animals were treated as described in Fig. 2 (n = 5; **P < 0.01). B: effect of the captopril on renal renin mRNA expression. Renin mRNA was detected by Northern blotting and normalized to GAPDH mRNA expression (n = 4; *P < 0.05). Inset: representative experiment. Lane 1: +/- without captopril treatment; lane 2: +/- with captopril treatment; lane 3: +/- with combined captopril and SC-58236 treatment; lane 4: −/− without captopril; lane 5: −/− with combined captopril and SC-58236 treatment; and lane 6: −/− with captopril. C: RRC in response to captopril treatment. Animals were treated as described in Fig. 2A. Renal renin activity was determined as described in MATERIALS AND METHODS (n = 5; **P < 0.01).
DISCUSSION

In the adult mammalian kidney, renin is synthesized by the JG cells, a group of myoepithelioid cells located in the afferent arteriole at the entrance to the glomerulus. The macula densa, a specialized segment of thick ascending loop of Henle located between the afferent and efferent arterioles, participates in regulation of JG renin secretion as well as tubuloglomerular feedback. Previous studies using nonselective COX inhibitors demonstrated a role for macula densa/cTAL-derived prostanoids in regulation of renin secretion and expression (16, 26, 41, 42, 47).

Administration of either ACEIs or AT₁-receptor antagonists results in increases in both renin mRNA and ir protein in the kidney in the absence of any detectable alteration in intravascular volume or renal hemodynamics (9, 15, 18). In previous studies, we determined that administration of an ACEI or an AT₁-receptor blocker led to significant increases in macula densa/cTAL COX-2 expression (11, 12). Furthermore, in rats treated with ACEIs, elevations in plasma and kidney renin were significantly inhibited by simultaneous treatment with a selective COX-2 inhibitor (11). In additional studies, we found that increases in renal renin expression in response to ACEI were significantly blunted in COX-2 knockout mice.

Studies in mice on sodium-deficient diets also indicated that the COX-2-selective inhibitor NS-398 would prevent renal renin expression (20); similar findings were observed in COX-2 −/− mice (51). Similarly, in humans on a sodium-deficient diet, PRA was significantly blunted by addition of the COX-2 inhibitor rofecoxib (28).

However, in contrast to the above studies, other investigators reported that treatment with COX-2-selective inhibitors did not affect renal renin expression and/or secretion. Harding et al. (19) reported that in rats, NS-398 decreased low salt-induced PRA but did not affect renal renin content. Kammerl et al. (29) also
reported that rofecoxib decreased PRA induced by low salt but not low salt plus an ACEI, and COX-2 inhibition did not alter renal renin content in any condition. This same group of Kurtz and co-workers (23) also reported that celecoxib did not affect increased renal renin mRNA levels induced by the AT1-receptor antagonist candesartan. Furthermore, in isolated perfused kidneys, they reported that NS-398 blocked increased renin secretion induced by bumetanide but not low-salt or ACE inhibition (10).

These divergent findings with COX-2 inhibitors coupled with previous reports by Kurtz and co-workers demonstrating that the nonselective, nonsteroidal anti-inflammatory drug indomethacin blunted increases in renal renin expression by either bumetanide or ACEIs (41, 43) raised the question of whether COX-1-derived prostanoids might be involved in regulation of renin. SC-58236 is a highly selective COX-2 inhibitor (39), but we could not absolutely rule out the possibility that there was also some inhibition of renal COX-1 in our previous studies. Furthermore, because COX-2 knockout mice have known renal developmental abnormalities (13, 32, 36), we could not completely rule out the possibility that the observed failure to increase renin production in response to ACEI was due to structural or functional abnormalities not directly related to the absence of COX-2 expression in macula densa/cTAL. To test definitively a potential role for COX-1 in ACEI-mediated increases in renin, we used mice with genetic deletion of the COX-1 gene. The fact that captopril increased plasma renin and renal renin mRNA and activity levels to the same extent in the COX-1 knockout and wild-type mice and the increased renin expression was effectively blocked by the selective COX-2 inhibitor SC-58236 is not involved in this process. EP4 mRNA is expressed in the glomerulus and collecting duct and may regulate glomerular tone (4, 5, 8). The current studies suggest that EP4 may also be involved in regulation of renal renin release. In this regard, EP4 transcripts in glomeruli were increased twofold by salt deprivation (26).

In conclusion, the current study provides definitive evidence that metabolites of COX-2 rather than COX-1 can mediate or modulate ACEI-induced renin production and release. The fact that EP2 null mice also demonstrated no alterations in ACEI-induced renin production and release suggests involvement of EP4 and/or IP.

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