Expression and function of COX isoforms in renal medulla: evidence for regulation of salt sensitivity and blood pressure

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Ye, Wenling, Hui Zhang, Elaine Hillas, Donald E. Kohan, R. Lance Miller, Raoul D. Nelson, Matthew Honegger, and Tianxin Yang. Expression and function of COX isoforms in renal medulla: evidence for regulation of salt sensitivity and blood pressure. Am J Physiol Renal Physiol 290: F542–F549, 2006. First published September 27, 2005; doi:10.1152/ajprenal.00232.2005.—Expression of cyclooxygenase (COX)-2, but not COX-1, in the renal medulla is stimulated by chronic salt loading; yet the functional implication of this phenomenon is incompletely understood. The present study examined the cellular localization and antihypertensive function of high-salt-induced COX-2 expression in the renal medulla, with a parallel assessment of the function of COX-1. COX-2 protein expression in response to high-salt loading, assessed by immunostaining, was found predominantly in inner medullary interstitial cells, whereas COX-1 protein was abundant in collecting duct (CD) and inner medullary interstitial cells and was not affected by high salt. We compared mRNA expressions of COX-1 and COX-2 in CD vs. non-CD cells isolated from aquaporin 2-green fluorescent protein transgenic mice. A low level of COX-2 mRNA, but a high level of COX-1 mRNA, as determined by real-time RT-PCR, was detected in CD compared with non-CD segments. During high-salt intake, chronic infusions of the COX-2 blocker NS-398 and the COX-1 blocker SC-560 into the renal medulla of Sprague-Dawley rats for 5 days induced ~30- and 15-mmHg increases in mean arterial pressure, respectively. During similar high-salt intake, COX-1 knockout mice exhibited a gradual, but significant, increase in systolic blood pressure that was associated with a marked suppression of urinary PGE2 excretion. Therefore, we conclude that the two COX isoforms in the renal medulla play a similar role in the stabilization of arterial blood pressure during salt loading.

cyclooxygenase-1; cyclooxygenase-2; mean blood pressure; prostaglandins; renal medullary interstitial cells

PROSTAGLANDINS (PGs) are important autocrine/paracrine factors that contribute to salt balance and blood pressure (BP) control through mechanisms that primarily involve the regulation of vascular tone and renal excretory function. The vascular endothelium is a rich source of prostacyclin (PGI2) and a potent vasodilator and inhibitor of platelet aggregation (5), whereas platelets produce thromboxane A2, a potent vasoconstrictor (9). The PGI2-thromboxane A2 balance is considered to play an important role in the maintenance of normal vascular tone. The kidney is capable of synthesizing all types of PGs, especially PGE2 and PGI2, which influence urinary sodium excretion directly through inhibition of tubular transport function and indirectly through regulation of activity of the renin-angiotensin system (4). Within the kidney, the inner medulla has the greatest capacity for PG synthesis. In vitro studies have demonstrated that PGs, such as PGE2, can directly inhibit NaCl transport in isolated thick ascending limbs (30) and collecting ducts (CDs) (11, 31). In addition, PGE1 augments renal medullary blood flow and attenuates the hydroosmotic effect of antidiuretic hormone (10), which, alone or in combination, promote urinary salt and water excretion. A large number of studies have documented that chronic salt loading stimulates the synthesis of PGE2 (19) and PGF2α (7, 34) from renal medullary cells. These observations are compatible with the notion that renal medullary PGs may serve as homeostatic regulators of sodium balance and BP.

Cyclooxygenase (COX), a rate-limiting enzyme in the PG biosynthesis pathway, exists in two major isoforms: the constitutive COX-1 and the inducible COX-2 (28, 29, 36, 37). Molecular cloning of these COX isoforms provides a novel tool to explore the physiological functions of renal PGs. Within the kidney, both COX isoforms are expressed at substantially higher levels in the inner medulla than in the cortex (3, 38, 40). Increases in the expression of renal medullary COX-2, but not COX-1, are seen in response to chronic salt loading (8, 15, 40). Emerging evidence suggests that renal medullary COX-2 may play a role in the promotion of sodium excretion and, thereby, the stabilization of BP (41). Major goals of the present study are as follows: 1) to resolve cellular localization of high-salt-induced COX-2 expression in the renal medulla, 2) to provide further evidence for the antihypertensive function of renal medullary COX-2 with use of chronic infusion and telemetry techniques, and 3) to examine the potential role of renal medullary COX-1 in BP regulation.

METHODS

Animals. Male Sprague-Dawley (SD) rats (250–300 g body wt) were obtained from Harlan Laboratories. COX-1 mutant mice were originally generated by Langenbach et al. (18), and this mouse colony was propagated at the University of Utah and maintained on a mixed 129-C57BL/6 background. All animal procedures were approved by the University of Utah Institutional Animal Care and Use Committee.

High-salt treatment for analysis of gene expression. Mice (3–4 mo old on a 129-C57BL/6 background) and SD rats were fed a normal- or high-salt diet for 14 days. Animals fed the high-salt (8% NaCl) diet and those fed the normal-salt (0.4% NaCl) diet had free access to tap water. At the end of experiments, with the animals under general anesthesia, the renal inner medullas were harvested and subjected to gene expression analysis.

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Genotyping. Genomic DNA was isolated from a short piece of tail taken at the time of weaning. Genotyping was determined by PCR with COX-1 gene-specific primers, which were positioned in the targeted region, and with Neo-specific primers. The sequences of the oligonucleotide primers were as follows: 5'-AGGAGATGGCTGCTGATTTGGGC-3' (bp 1496–1516, sense) and 5'-AATCTGACTTCTTGAGTTGCC-3' (bp 2077–2097, antisense) for COX-1 (accession no. 200302) and 5'-CTTGGGAGAGAGGCTATTC-3' (bp 191–210, sense) and 5'-AGGTGAGATGACAGAGGATC-3' (bp 451–470, antisense) for Neo. Amplification was carried out for 30 cycles of 94°C for 40 s, 58°C for 40 s, and 72°C for 40 s and a final extension at 72°C for 8 min.

Experiments in COX-1 knock-out mice. Systolic BP (SBP) in mice was determined by the tail-cuff method with a BP analysis system (model BP2000, Visitech, Apex, NC) (17). Adult male COX-1−/− and COX-2−/− mice (3–4 mo old) were habituated to the BP measurement device for 7 days. These mice were fed a normal-salt diet for 3 days and then a high-salt diet for 8 days. Two cycles of 20 measurements of SBP were recorded per day for the entire experimental period. To eliminate variations between the four different channels on the platform, all measurements were conducted using the same channel. The animals were placed in metabolic cages before and after high-salt treatment for collection of 24-h urine specimens.

Immunohistochemistry. Kidneys from the normal- and high-salt-fed SD rats were perfusion fixed with 3% paraformaldehyde through an aortic cannula and then processed for frozen sectioning. Cryostat sections (5 μm thick) were incubated in PBS containing 0.5% Triton X-100 for 30 min. After the sections were rinsed in PBS, unspecific protein binding sites were blocked by 2 h of incubation in 5% dry milk. Primary antibodies [rabbit anti-COX-2 (catalog no. 160106) and rabbit anti-COX-1 (catalog no. 160109)] and Cy2-labeled secondary antibody were added in a 1:50 dilution in 5% dry milk, and the sections were incubated overnight at room temperature. After the sections were rinsed in PBS, signals were detected with a Cy2-labeled secondary antibody and viewed in an Olympus IMT-2 microscope with a fluorescence module.

Western blotting. Renal inner medullas from the salt-manipulated animals were lysed and subsequently sonicated in PBS that contained 1% Triton X-100, 250 μM PMSF, 2 mM EDTA, and 5 mM DTT (pH 7.5). Protein concentrations were determined by the use of Coomassie reagent. Protein (40 μg) for each sample was denatured in boiling water for 10 min, separated by SDS-PAGE, and transferred to nitrocellulose membranes. The blots were blocked overnight with 5% nonfat dry milk in Tris-buffered saline and then incubated for 1 h with the COX-2 polyclonal antibody. After they were washed with Tris-buffered saline, the blots were incubated with goat anti-rabbit horseradish peroxidase-conjugated secondary antibody and visualized with enhanced chemiluminescence.

Enzyme immunoassay. The inner medullas from the salt-manipulated SD rats were homogenized with a Polytron and then subjected to sonication. PGs were extracted with ethanol and then purified with C-18 cartridges (Sep-Pak Classics). Urine samples were centrifuged at 200 × g for 5 min at 10,000 rpm and diluted 1:1 with enzyme immunoassay buffer. Concentrations of PGE2 and PGF2α were determined by enzyme immunoassay (Cayman Chemicals).

Fluorescence-assisted microdissection and real-time RT-PCR. CDs were isolated from whole kidneys of transgenic mice that expressed the aquaporin (AQP) 2 green fluorescent protein (GFP) transgene (24). Briefly, both mouse kidneys were removed and placed in ice-cold Krebs solution, minced with a razor blade, and placed in 4 ml of Krebs digestion medium at 37°C for 20–30 min, with gentle vortex mixing every 5 min. The tubular digest was washed by addition of 6 ml of sterile water to each 4 ml of tubular digest and then centrifuged at 200 g for 5 min. The tubular digest was resuspended in 5 ml of ice-cold PBS that contained 1% BSA. One drop of tubular fragments was diluted in 5 ml of ice-cold PBS that contained 1% BSA and then placed in a 5-cm plastic petri dish on a inverted fluorescence/bright-field microscope for fluorescence-assisted microdissection. CDs and non-CDs were isolated by aspiration with a micropipette that was held in place by an in-house-constructed motor-driven (model 860A, Newport, Irvine, CA) micromanipulator (model 8537, Narishige). Each micropipette was pulled to a 200- to 250-μm-ID tip with a 45° angle from a 6-inch-long fire-polished glass capillary (model N-51-A, Drummond Scientific, Broomall, PA) with outer and inner diameters of 0.084 and 0.064 inches, respectively. Total RNA from fluorescence-assisted microdissected tubules (12 μl) was isolated using the RNeasy Mini or RNeasy Micro Column with DNase I treatment (Qiagen) according to the manufacturer’s recommendations. Total RNA (2.5 μg from organs, 6 μl from the RNeasy Micro Column, and 12 μl from the RNeasy Mini Column) was reverse transcribed with oligo(dT) using Superscript Reverse Transcriptase II (Invitrogen) according to the manufacturer’s recommendations.

For real-time PCR, oligonucleotides were chosen by the Primer Express 1.0 (PE Applied Biosystems) with probes positioned at an exon-intron junction (25). Sequences of oligonucleotides were 5′-CCTGGAAGCGTACACATCA-3′ (sense) and 5′-GGTGACCTGAGGCTCTTCAATTT-3′ (antisense) for COX-2 and 5′-GAGTTGAGGACAGAGGATC-3′ for Neo. Amplification was performed using the TaqMan Universal PCR Master Mix and the Prism 7900 Sequence Detection System (Applied Biosystems). Cycling conditions were 50°C for 2 min and 95°C for 10 min followed by 40 repeats of 95°C for 0.15 min and 60°C for 1 min. Relative amounts of mRNA, normalized by 18S rRNA, were calculated from threshold cycle numbers (CT, i.e., 2−ΔΔCT) according to the manufacturer’s suggestions.

Chronic intramedullary and intravenous infusion protocols. Chronic studies were conducted in normotensive SD rats. To eliminate compensatory changes in the contralateral kidney, the right kidney was removed from all rats. At the same time, the animals were instrumented for telemetry measurements of BP. After 1 wk of recovery, a second surgery was performed for implantation of a renal interstitial catheter into the renal medulla according to the published protocol (21). Briefly, the tip of the interstitial catheter was placed in the renal medulla by a 3- to 4-mm insertion into the kidney through a small hole made by a 26-gauge needle. The catheter tip was anched to the kidney surface with Microsilk surgical mesh and Vetbond tissue adhesive. The tubing was attached to the kidney, forced through the muscle layer, exteriorized to the dorsal nape of the neck, and connected to a swivel apparatus that allowed the rats to move freely without tangling the catheters. After the surgical procedures, each rat was housed individually in a metabolic cage and received a continuous medullary interstitial infusion of saline at a rate of 8 μl/min via a high-power infusion pump (Harvard). The animals were allowed tap water ad libitum and were fed a low-sodium (0.03% NaCl) or high-sodium (8% NaCl) diet. After recording of daily mean arterial pressure (MAP) measurements during the 2-day control period, the COX-2 blocker NS-398 (10 mg·kg−1·day−1) or the COX-1 blocker SC-560 (7.5 mg·kg−1·day−1) was added to the interstitial infusate. These doses were chosen because of their minimal effects on BP when administered via intravenous infusion. Both drugs were dissolved in the same organic solvent, which consisted of 0.7% DMSO and 0.99% Tween 80. Infusion of this solvent alone served as the vehicle control. The placement of the catheter in the renal medulla was examined at the end of each study, and any animals with inappropriate placement of the catheter or necrosis of the renal medulla were removed from the study.

To control for spillover of each drug into the circulation, an additional group of rats was infused intravenously with NS-398 or SC-560 at the same dose and at the same infusion rate (8 μl/min) used for intramedullary infusion. The intravenous catheter was placed in...
the jugular vein. Intravenous infusion control has been widely used to validate the effectiveness of site-specific actions of locally infused agents (21, 33).

Telemetry measurements of BP. Telemetry was implanted through the catheterization of the carotid artery. Briefly, under general anesthesia, a catheter was placed in the carotid artery, and the transmitter body that connected to the catheter was placed under the skin in the ventral abdominal area according to the manufacturer’s instructions (model TA11PA-C40, DSI). The animals moved freely, and 24-h BP measurements were recorded.

Statistical analysis. Statistical analysis for BP measurements was performed by ANOVA and Bonferroni’s test. Densitometry, PGF$_{2\alpha}$ and PGE$_2$ values, and COX isoform mRNAs were analyzed by paired or unpaired Student’s $t$-test. Values are means ± SE. $P < 0.05$ was considered statistically significant.

RESULTS

High-salt stimulation of COX-2 expression in the renal inner medulla. Immunoblotting for COX-1 and COX-2 in the kidneys of SD rats fed a normal- or high-salt diet was performed. Rats in normal- and high-salt groups were fed a diet containing 0.4% NaCl and 8% NaCl, respectively, for 14 days. Figure 1 shows immunostaining of the two COX isoforms in the renal medulla. A remarkable induction of COX-2 expression in the high-salt group was detected predominantly in renal medullary interstitial cells (RMICs). COX-1 protein was detected in CD and interstitial cells and was not significantly affected by high salt. After high-salt treatment, the production of PGF$_{2\alpha}$ and PGE$_2$ in the renal inner medulla was significantly increased (Fig. 2). Renal medullary COX-2 response to high salt was also examined in mice. Chronic salt loading increased renal medullary COX-2 protein expression to a similar extent in the mice and the rats (Fig. 3) (40).

COX-2 mRNA expression in GFP-labeled CD isolated from AQP2-GFP transgenic mice. Because of the conflicting reports concerning COX-2 expression in CDs, we compared COX-2 mRNA expression in CD vs. non-CD tubules isolated from transgenic mice expressing the AQP2-GFP transgene. GFP expression was restricted to principal cells but not intercalated cells. The isolated GFP-expressing cells have been shown to express principal cell markers, such as AQP2, AQP3, and vasopressin type 2 receptor, but not the intercalated cell marker H$^+$-ATPase (42). In the present study, the GFP-positive and GFP-negative tubules (CD vs. non-CD tubules) were isolated by the fluorescence-assisted microdissection technique. mRNA expressions of COX-1 and COX-2 were determined by real-time RT-PCR. $C_T$ values in CD vs. non-CD groups were $31.5 \pm 0.6$ vs. $34.5 \pm 0.4$ for COX-1, $36.1 \pm 0.5$ vs. $29.7 \pm 1.2$ for COX-2, and $22.9 \pm 0.4$ vs. $22.1 \pm 0.3$ for 18S RNA ($n = 4$). Relative amounts of mRNA, normalized by 18S rRNA, were calculated from the $C_T$ values (i.e., $2^{-\Delta\Delta C_T}$). A low level of COX-2 mRNA, but a high level of COX-1 mRNA, was detected in the CD compared with the non-CD tubules (Fig. 4).

Effect of chronic inhibition of COX-2 in rat renal medulla on BP. Uninephrectomized SD rats were infused with NS-398 (10 mg·kg$^{-1}$·day$^{-1}$) through a catheter that was implanted in the renal medulla, and BP was monitored by telemetry. NS-398 was intravenously infused at the same rate to control spillover. In another control group, vehicle was continuously infused into the renal medulla. All animals were fed a high-salt (8% NaCl) diet for the entire experimental period. MAP increased gradually after the intramedullary infusion of NS-398 and, to a lesser extent, after intravenous infusion of the drug (Fig. 5). After 5 days of intramedullary infusion of NS-398, MAP increased from $120.9 \pm 3.4$ to $152.7 \pm 7.5$ mmHg. Intravenous infusion
of NS-398 at the same dose and same infusion rate induced a small, but significant, increase in MAP. After 5 days of intravenous infusion of NS-398, MAP increased from 114.1 ± 2.0 to 124.1 ± 4.6 mmHg. The increases were much greater in the intramedullary NS-398 group than in the intravenous NS-398 group (P < 0.05). Continuous infusion of vehicle into the renal medulla had no significant effect on MAP.

Assessment of BP in SD rats receiving intramedullary infusion of SC-560 and in salt-loaded COX-1 knockout mice. Despite the high level of COX-1 expression in the renal medulla, the function of renal medullary COX-1 largely remains elusive. The present study examined BP in SD rats chronically infused with the COX-1 inhibitor SC-560 through an implanted catheter in the renal medulla, as well as in COX-1 knockout (KO) mice. Rats that were subjected to chronic intramedullary infusion of SC-560 and fed a high-salt diet exhibited a gradual and significant increase in MAP (Fig. 6). After 5 days of intramedullary infusion of SC-560 and a high-salt diet, MAP increased from 120.7 ± 3.0 to 135 ± 3.2 mmHg (P < 0.01). This result was completely prevented by a low-salt diet. During high-salt feeding, neither intravenous infusion of SC-560 nor intramedullary infusion of vehicle had a significant effect on MAP.

SBP in COX-1 wild-type mice, measured by the tail-cuff method, did not change significantly in response to the high-salt diet for 1 wk. In contrast, after this treatment, SBP gradually and significantly increased in COX-1−/− mice (Fig. 7A). After 1 wk of high-salt treatment, SBP increased from 102.4 ± 2.8 to 128.0 ± 4.0 mmHg (P < 0.01) in these mice. After high-salt treatment, the wild-type mice exhibited a marked increase in 24-h urine PGE2 output (734.8 ± 78.3 vs. 3,238.2 ± 644.3 pg/24 h, P < 0.05). In contrast, this increase in urinary PGE2 excretion was remarkably attenuated in COX-1−/− mice (656.1 ± 137.2 and 1,111.6 ± 66.2 pg/24 h for normal and high salt, respectively, P < 0.05).

DISCUSSION

Our previous study demonstrated that chronic salt loading induced a remarkable increase in COX-2 mRNA and protein (~10 fold) levels in the renal inner medulla of SD rats while COX-1 expression was unaffected (40). The present study extends this observation by further investigation of the expression and function of renal medullary COX isoforms in response to chronic salt loading. We demonstrate that, despite the differences in the cellular localization and regulation, both COX isoforms are involved in the stabilization of BP during high-salt intake.

The response of renal medullary COX-2 to high-salt treatment was initially observed in rats. The same response was observed in mice in the present study, suggesting that the phenomenon is universal across different species. Using immunostaining techniques, we, for the first time, determined the cellular localization of high-salt-induced COX-2 expression in the rat renal inner medulla. Consistent with the immunoblotting data, chronic salt loading induced a marked increase in COX-2 protein expression in the renal medulla that was primarily localized to RMICs. This localization characteristic is compatible with the observation that inhibition of COX-2...
reduces renal medullary blood flow (26). RMICs are an important source of vasoactive hormones, notably PGs and nitric oxide, through which RMICs exert an influence on renal medullary blood flow and tubular transport, thereby regulating sodium balance and BP. In contrast to the cellular localization pattern of COX-2, COX-1 expression was found in renal medullary epithelial cells as well as RMICs and was unaffected by salt loading, consistent with the previous observation (40).

Previous reports concerning COX-2 expression in the CD are conflicting. Immunohistochemistry studies document dominant (1, 23, 27, 39) or no expression in the CD (6, 12, 13). Differences in sources of antibodies and types of tissue sections might account for some of these variations. It is imperative to address this issue using independent techniques. We took advantage of currently available AQP2 promoter-GFP transgenic mice to determine COX-2 expression in a relatively pure population of the CD segment. COX-2 mRNA was detected at much lower levels in the GFP-positive CD than in the GFP-negative tubular segments. These observations substantiate the difference in the renal expression pattern of the two COX isoforms.

To address the potential roles of renal medullary COX isoforms in the regulation of BP, we examined the effects of chronic intramedullary infusion of the COX-2 blocker NS-398 and the COX-1 blocker SC-560 on BP. A 5-day intramedullary infusion of NS-398 and SC-560 into salt-loaded rats resulted in -30- and 15-mmHg elevations of MAP, respectively. As a common way to control spillover, a separate group of rats was intravenously infused with these drugs at the same doses and same infusion rates used for intramedullary infusion. Intravenous infusion of NS-398 or SC-560 had only a modest effect or no effect on MAP. The increases in MAP were much greater in the groups subjected to intramedullary infusion of NS-398 and SC-560 than in intravenous control groups. This finding indicated that the hypertensive effects of NS-398 and SC-560 were likely due to their local actions in the renal medulla. Because of poor water solubility, both agents were dissolved in 0.7% DMSO and 0.99% Tween 20-PBS. Chronic infusion of this organic solvent into the renal medulla did not significantly affect MAP and, thus, ruled out any influences of the vehicle on renal medullary function. Selectivity of the two compounds at the dose used remains a concern, inasmuch as local tissue concentrations are unknown. Metabolic studies were attempted to estimate sodium balance and urinary PGE2 output but were unsuccessful because of technical problems. Because of the presence of the telemetry transmitter in the abdomen and

![Fig. 4. mRNA expression of COX-1 and COX-2 in isolated green fluorescent protein (GFP)-labeled CD and non-CD segments (n = 4 in each group). GFP-labeled CD and GFP-negative segments from kidney of aquaporin 2 (AQP2)-GFP transgenic mice were microdissected under a stereomicroscope. mRNAs of COX-1 and COX-2 were determined by real-time RT-PCR and normalized by 18S rRNA.](image-url)

![Fig. 5. Effects of intramedullary infusion of NS-398 on mean arterial pressure (MAP). At 1 wk after right uninephrectomy, adult Sprague-Dawley rats received intramedullary (n = 7) or intravenous (n = 6) infusion of vehicle or NS-398 at the same dose (10 mg·kg⁻¹·day⁻¹) and same infusion rate (8 μl/min) for 1–7 days. A separate group of animals received intramedullary infusion of vehicle for the entire experimental period (n = 10). Chronic intramedullary and intravenous infusions were performed through implanted catheters in renal medulla of the remaining kidney and jugular vein, respectively. All animals were fed the high-salt diet for the entire experimental period. Telemetry was used to monitor 24-h MAP. *P < 0.05; #P < 0.01 vs. day 2 vehicle within the same group; ^P < 0.05; –P < 0.01 vs. intravenous NS-398 and intramedullary vehicle for the corresponding period.](image-url)
infusion catheters in the neck, the rats were unable to reach the food placed outside the metabolic cage.

Observations of the chronic infusion studies, conducted independently by Zewde and Mattson (41) and us, were similar: site-specific inhibition of COX-2 in the renal medulla produces hypertension in animals fed a high-salt diet, despite a few differences in the experimental protocols (telemetry vs. the arterial catheterization method for BP measurement and 8% NaCl vs. 4% NaCl). The two studies are mutually supportive.

In support of the observation made with chronic infusion of the COX-1 inhibitor SC-560 into the renal medulla produced salt-sensitive hypertension, suggesting natriuretic and antihypertensive functions of renal medullary COX-1. Consistent with this notion, >50 clinical trials that involve 13,000 subjects have shown that edema is among the most common side effects of COX-2 inhibition (35). Systemic administration of COX-2 inhibitors to experimental animals increases BP in a salt-dependent manner (14, 22).

Compared with the detailed knowledge about renal COX-2, the function of renal COX-1 largely remains elusive. Using the chronic infusion and telemetry techniques, we demonstrated that chronic infusion of the COX-1 blocker NS-398 or the COX-1 blocker SC-560 into the renal medulla produces hypertension in animals fed a high-salt diet. However, inconsistencies between the two studies exist in the differences in urinary PGE2 excretion. Kawada et al. found a reduced urinary excretion of PGE2, as well as other types of PGs, during normal-salt feeding in COX-1 KO mice. However, we found comparable urinary PGE2 levels under basal conditions but no significant increases in urinary PGE2 excretion after high-salt treatment in the KO mice, as seen in the wild-type controls. The reason for such inconsistency is not clear and could be related to differences in experimental protocols. Athirakul et al. (2) observed a reduction in BP in COX-1 KO mice during low-salt feeding with increased sodium loss.

In summary, the present study extends our previous observations that chronic salt loading induces COX-2, but not COX-1, expression in the rat renal inner medulla. Immunohistochemistry shows high-salt-induced COX-2 expression predominantly in RMICs, in contrast to the unchanged COX-1 expression in CD and RMICs. Chronic intramedullary infusions of the COX-2 blocker NS-398 or the COX-1 blocker...
SC-560 significantly elevate MAP in a salt-loading state. During high-salt feeding, COX-1 KO mice develop hypertension, with a marked suppression of urinary PGE2 excretion. Collectively, these studies will contribute to a better understanding of COX-dependent mechanisms in the regulation of sodium balance and BP.

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