Supplement Material

Animal husbandry Animals were maintained on a 12-hour light/12-hour dark cycle with normal mouse chow (0.7% NaCl) and water provided ad libitum. As for high salt challenge, mice were fed with 8% NaCl chow diet for 2 weeks. All COX-1>COX-2 and COX-2 null mice used for the experiments were initially produced on a mixed C57BL/6 x Sv129 genetic background (50%:50%). Both genotypes had been individually maintained using a heterozygous by heterozygous cross breeding strategy for more than 20 generations.

The effect of Cicaprost on salt sensitive hypertension in mice 8-10 week old COX-1>COX-2 mice were randomly divided into two groups. One group was administered Cicaprost (50 µg/kg/d in 10 mM Tris/0.15 M NaCl solution, provided by Bayer Schering Pharma AG) subcutaneously via Alzet osmotic mini-pumps (model 2004) as described previously\(^1\), another group received vehicle solution as a control (10 mM Tris/0.15 M NaCl solution). A HSD (8% NaCl) was initiated after implantation of osmotic mini-pumps and fed for an additional two weeks; blood pressure was recorded routinely by tail-cuff before mini-pump implantation and after HSD.

RMIC cell culture RMICs were isolated from male mice (10 weeks old) using a standard method described by Fontoura et al\(^2\). Medullary regions of kidney containing inner medulla and inner zone of the outer medulla were dissected. The dissected tissue was finely minced in Hanks’ balanced salt solution (HBSS), then was digested with 170 unit/ml collagenase type I for 45 min at 37°C (Washington Biochemical). The digested tissue were further sieved through 105 um mesh before re-suspended in a 1:1 mixture of culture medium RPMI 1640 (20% fetal calf serum, 0.66 U/ml insulin, 2 g/l NaHCO\(_3\), 15mM Hepes, 250 ug/l amphotericin B) and Dulbecco’s modified Eagle’s medium (DMEM: 10% fetal calf serum, 3.7 g/l NaHCO\(_3\), 0.66 U/ml Insulin, 15mM Hepes, 250 ug/l amphotericin B) conditioned by 3T3 Swiss albino mouse fibroblasts in the log phase of growth. Cells were maintained at 37°C in 95% O\(_2\)-5% CO\(_2\) incubator. These cells exhibited characteristic abundant oil red-O–positive lipid droplets. Cells were typically studied at their 3rd and 4th passages.

Culture medium osmolality could be modulated by adding extra NaCl and mannitol. The final concentration of NaCl and mannitol at 630 mOsm/kg.H\(_2\)O and 930 mOsm/kg H\(_2\)O
are 0.08 M and 0.1 M, 0.16 and 0.2, respectively. As for low osmolality of 230 mOsm/kg.H2O, the culture medium was diluted 2:3 with distilled water. Once the desired osmolality was achieved, the cells were incubated for 24 h for further experiment.

**Primary endothelial cell (EC) culture** ECs were prepared from 6 week-old mice as previously discribed\(^3\). Briefly, lungs from two mice each group were harvested, minced finely and digested in collagenase (Worthington Biochemical Co, 200 U/ml) at 37°C for 45 minutes. After dissociated by titrating and filtered through a 100 μm disposable cell strainer (Becton Dickinson Labware), the lung cells were incubated with PECAM-1-coated beads (ratio: 1.5μg antibody to 10⁷ beads) for 20 min at 4°C, then recovered by magnetic separator and seeded in complete culture medium in 60 mm dish. When the cells reached 70 to 80% confluence (around 3 days), a 2nd sort by ICAM-2-coated beads was applied to get 99% EC purity\(^4\). Passage 3 cells were used for co-culture experiments.

**RMIC/EC transwell co-culture and cellular cAMP Measurement** \(5 \times 10^4\) RMICs and \(3 \times 10^5\) ECs each well were seeded in Transwell Insert (0.4 um pore, 12 Well, Corning Incorporated) and regular 12-well plate (Corning Incorporated), respectively. Indomethacin (10μM, Sigma) was included overnight in the EC medium to block endogenous PGs before co-culture. RMICs were changed to high osmolality medium (630 mOsm/kg.H₂O) or containing 5 μg/ml LPS for 5 hours to upregulate COX-2 expression, then placed over EC minelayers (1.5 ml/well serum-free medium containing 1 mM isobutylmethylxanthine (IBMX), phosphodiesterase inhibitoir) and incubated for additional 2 hours. The serum-free medium was collected for PG analysis; cellular cAMP in cultured EC was extracted with ice-cold 65% ethanol for 30 min and then quantified using a radioimmunoassay kit (Amersham) according to the manufacturer’s instructions.

**Western blotting** Cultured RMICs were washed with PBS and harvested in NuPAGE lysis buffer (invitrogen) followed by repetitive aspiration using a 27-gauge needle. Protein (10 μg) was loaded into each lane, separated on 4-10% BisTris-NuPAGE gels (Invitrogen) and transferred to Hybond ECL nitrocellulose membranes (Amersham Biosciences). Rabbit anti-COX-1 polyclonal antibody (Cayman Chemical Co.) at a 1:500 dilution, rabbit anti-COX-2 polyclonal antiserum (Cayman Chemical Co.) at a 1:1000 dilution, Rabbit anti-IP receptor polyclonal antibody (Cayman Chemical Co.) at a 1:500
dilution, and mouse anti-β-actin monoclonal antibody (Sigma) at a 1:5000 dilution were used as primary antibodies. Horseradish peroxidase-conjugated goat anti-mouse IgG (Sigma) at 1:5000, and horseradish peroxidase-conjugated goat anti-rabbit IgG (Sigma) at a 1:2000 dilution, were used as secondary antibodies, respectively. Signals were detected by ECL (Amersham Biosciences).

**PG extraction and measurement** Slices of inner medulla were carefully dissected and homogenized in 1 ml of ice cold PBS containing 100μM indomethacin using stainless steel beads (Qiagen). Residual tissue was separated by centrifugation, and the supernatant was collected. For PGs from cultured RMICs, cells were incubated with 30 μM arachidonic acid (AA, Cayman Chemical Co.) in PBS for 15 minutes. Either the supernatant from fresh tissues or culture medium was spiked immediately with 5ng PGD₂-d₄ (Cayman Chemical Co, Cat# 312010), TxB₂-d₄ (Cayman Chemical Co, Cat# 319030), 6-keto Prostaglandin F₁α-d₄ (Cayman Chemical Co, Cat# 315210), PGF₂α-d₄ (Cayman Chemical Co, Cat# 316010), PGE₂-d₄ (Cayman Chemical Co, 314010), then purified by solid phase extraction using StrataX C18 cartridges (Phenomenex). The solid phase extraction cartridge was conditioned with 1 ml of acetonitrile and equilibrated with 1 ml of water. The sample was applied to the cartridge, which was then washed with 1 ml of 5% acetonitrile in water and dried with vacuum for 15 min. The analyte and internal standards were eluted from the cartridge using 1 ml of 5% acetonitrile in ethyl acetate. The eluate was collected and dried under a gentle stream of nitrogen. The resulting residue was reconstituted in 200 μl of 5% acetonitrile in water and filtered by centrifugation using 0.2-μm Nylon Microspin filters (Alltech Associates), then quantitated utilizing liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) analyses as described previously⁵.

**Urinary PG metabolite analysis** 24-hour urines from male mice (8-10 weeks) were collected using metabolic cages and prostanoid metabolites were extracted and quantitated as previously described⁶.

**RNA isolation.** Total RNA was extracted from tissues using RNeasy Mini-Kit (Qiagen). Reverse transcription was carried out on 400ng of RNA using Taqman Reverse transcription reagents (Applied Biosystems). The resulting cDNA was used for quantitative real time PCR.
Quantitative real time PCR TaqMan gene expression assays (Applied Biosystems, Foster City, Calif; catalog No. 4331182) for RENIN (Mm02342889_g1), COX-1 (Mm01336806_m1), COX-2 (Rn00568225_m1), EP1 (Mm00443098_g1), EP2 (Mm00436051_m1), EP3 (Mm00441045_m1), EP4 (Mm0043053_m1), FP (Mm00436055_m1), IP (Mm00801938_m1), TP (Mm00436917_m1), DP1 (Hs00235003_m1), DP2 (Rn00824628_m1) were performed on an ABI Prism 7900 Sequence Detection System. Results were normalized with 18S rRNA (Hs99999901_s1).

Histopathological Analysis Kidney and intestine were fixed in 10% buffered formalin for 24 h, processed routinely, and embedded in paraffin and stained with hematoxylin and eosin routinely.

Plasma BUN and creatinine Analysis Blood collected from the saphenous vein was analyzed for BUN levels by the clinic laboratory of the Veterinary Hospital of University of Pennsylvania.

Online Figure I. Comparison of blood pressure of WT mice from COX-1>COX-2 heterozygous mating and COX-2 heterozygous mating at normal chow diet and high salt diet. Mice (6 weeks old) were fed either normal diet (0.7% NaCl) or high-salt diet (8% NaCl) for 2 weeks, blood pressure was measured by tail-cuff method. Wt Ctl, Wild type controls. N=10-11, p=n.s.
Online Figure II. Normal renal function in COX-1>COX-2 mice after high salt intake.
Mice (6-7 weeks old) were fed high salt diet (8% NaCl) for 4 weeks, plasma and kidney samples were collected for renal function analysis. A. Plasma blood urea nitrogen (BUN) and creatinine in WT, COX-1>COX-2 and COX-2 KO mice. *P<0.05 vs COX-1>COX-2 and WT mice, n=5-8. B. Representative light photomicrographs of HE stained kidney sections from WT, COX-1>COX-2 and COX-2 KO mice after high salt treatment. Upper panel, 20X magnification; Lower panel, 400X magnification. Dotted lines represent the thickness of cortex. Black arrow, hypoplastic glomeruli near capsular surface.
Online Figure III. Effect of high salt intake on PG receptor expression in inner medulla region in COX-1>COX-2, COX-2 and WT mice. EP receptors (A, B, C, D), DP2 (E), FP (F) and TP (G) mRNA level in medulla from COX-1>COX-2, COX-2 KO and WT mice were quantitated by real time RT-PCR; HS, High salt diet treatment. *, p<0.05 vs WT controls, n=4-6
Online Figure IV. TP (A) and IP (B) expression in renal cortex of COX-1>COX-2, COX-2 KO and WT mice before and after high salt treatment. TP and IP receptor in renal cortex from COX-1>COX-2, COX-2 and WT mice were quantitated by real time RT-PCR; HS, High salt diet treatment (2 wks). *, p<0.05 vs WT controls, n=4-6.
Online Figure V. The effect of Cicaprost on salt induced hypertension in COX-1>COX-2 mice. COX-1>COX-1 mice were infused subcutaneously either Cicaprost (50μg/kg/d in 10 mM Tris/0.15 M NaCl solution) or Vehicle (10 mM Tris/0.15 M NaCl solution) through Alzet osmotic mini pimps, and then subject to High salt diet (HSD) for 2 weeks. Blood pressure was recorded before implantation (Normal chow diet) and after HSD treatment. *, p<0.01 vs Normal diet; #, p<0.01 vs Vehicle group, n=9.
Online Figure VI. RMIC COX2 derived PGI₂/IP signaling was impaired in cocultured vascular endothelial cells (ECs). RMICs in Transwell Inserts (Corning Incorporated) was sustained in either 330 or 630 mOsm/kg H₂O culture medium for 5 hrs, then co-incubated with 1 mM isobutylmethylxanthine (IBMX) pretreated primary ECs for additional 2 hrs. The EC media were subjected to PG analysis (A) and cellular cAMP was assayed in cultured ECs (B). Cica, 1μM Cicaprost was used directly in IBMX-treated ECs medium as a positive control for IP activation. *, p<0.05 vs WT group; #, p<0.01 vs 330 mOsm group, n=6.