Biomechanical regulation of cyclooxygenase-2 in the renal collecting duct

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1Department of Medicine, Icahn School of Medicine at Mount Sinai, New York, New York; 2Department of Medicine, The James J. Peters Veterans Affairs Medical Center, New York, New York; and 3Department of Pediatrics, Icahn School of Medicine at Mount Sinai, New York, New York

Submitted 10 June 2013; accepted in final form 29 October 2013

Liu Y, Flores D, Carrizoza-Gaytán R, Rohatgi R. Biomechanical regulation of cyclooxygenase-2 in the renal collecting duct. Am J Physiol Renal Physiol 306: F214–F223, 2014. First published November 13, 2013; doi:10.1152/ajprenal.00327.2013.—High-dietary sodium (Na), a feature of the Western diet, requires the kidney to excrete ample Na to maintain homeostasis and prevent hypertension. High urinary flow rate, presumably, leads to an increase in fluid shear stress (FSS) and FSS-mediated release of prostaglandin E2 (PGE2) by the cortical collecting duct (CCD) that enhances renal Na excretion. The pathways by which tubular flow biomechanically regulates PGE2 release and cyclooxygenase-2 (COX-2) expression are limited. We hypothesized that FSS, through stimulation of neutral-sphingomyelinase (N-SM) activity, enhances COX-2 expression to boost Na excretion. To test this, we used a co-culture system to study the effects of tubular flow and FSS on PGE2 release by CCD. We found that: 1) tubular flow and FSS stimulate a coordinated response to enhance PGE2 release: acutely, to activate cPLA2 and release arachidonic acid (AA), the substrate of COX-1/2, and 2) chronically, to induce COX-2 gene and protein expression so as to augment capacity of cyclooxygenases to metabolize AA into intermediate endoperoxides and, finally, into PGE2. These acute and chronic processes, we believe, are coordinated to ensure appropriate amounts of paracrine PGE2 are synthesized to excrete Na and maintain Na homeostasis. In this study, we sought to extend our initial findings on the effect of tubular flow and FSS on PGE2 expression by evaluating the in vivo and in vitro effects of tubular flow and FSS, respectively, on the regulation of COX-2 protein expression through a sphingomyelinase-dependent mechanism. Sphingomyelinases are enzymes that convert sphingomyelin to ceramide and this compound, in turn, acts as an intracellular signaling molecule. Sphingomyelinases are principally composed of two types: 1) acidic and 2) neutral sphingomyelinas (N-SM). N-SM enzymatic activity has been reported by others to be stimulated by FSS in endothelial cells and to induce COX-2 protein expression (4, 5, 27). Acid and N-SM activity have been reported in whole kidney, renal cortex, and, more specifically, proximal tubule (12, 37). N-SM is also observed in the mechanosensitive mesangial cell (9); however, the expression of N-SM in the CD has not been described.

MATERIALS AND METHODS

Cell culture. Murine immortalized inner medullary CD3 (IMCD3) cells were grown in DMEM/F12 (with 10% FBS) on 25 × 75-mm slides and studied when they reached confluence between 3 to 7 days. We only used cells up to passage 10 due to the risk of genetic drift.

Induction of FSS. Cells grown on slides were placed in laminar flow chambers (GlycoTech manufactured chamber), maintained at 37°C, and subject to shear of 0.4 dyn/cm² using phenol red-free, serum-free DMEM/F12 containing penicillin/streptomycin for varying durations. FSS was calculated based on Poiseulle’s law: μ = γ = 6μ/Q(a/b² where μ = wall stress (dyn/cm²), γ = shear rate (per s), μ = apparent viscosity of the fluid (media at 37°C = 0.76 cP), a = channel height (cm), b = channel width (cm), and Q = volumetric rate (ml/s). The DMEM/F12

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was not recirculated into the perfusion chamber. Static control cells were exposed to the same solution and duration as sheared cells, but without exposure to FSS. One milliliter of serum- and phenol red-free DMEM/F12 was incubated with either static or sheared cells for 1 h for measurement of PGE2 secretion (8, 17). Cells from the Glycotech chamber were then collected for total protein or for measurement of sphingomyelinase activity.

**Sphingomyelinase activity.** IMCD3 cells were processed and assayed according to the directions specified in the Sphingomyelinase Fluorometric Assay Kit (Cayman Chemical). In short, static or sheared cells were collected and washed in PBS; cells were then gently lysed in SMase buffer with a glass dounce and centrifuged. The supernatant and pellet were individually collected and frozen at −80°C for analysis. A standard curve was generated using phosphocholine (0 to 50 μM), a positive control containing sphingomyelinase, and background well containing SMase buffer solution alone. The samples derived from cells were then plated into wells. Developer (100 μl) was pipetted into each well and 20 μl of sphingomyelin substrate was added (except into the background wells). The plate was incubated for 30 min at 37°C and then fluorescence was measured in a SpectraMax M2 fluorescence plate reader (535-nm excitation and 590-nm emission; Molecular Devices).

**Western blotting.** Western blot analysis was performed as previously described (7). Protein lysates were generated from kidney or IMCD3 cells by incubating and homogenizing tissue in lysis buffer (10 mM Tris, pH 7.2, 1 mM EGTA, 1 mM EDTA, 150 mM NaCl, 0.5% NP-40, 1.0% Triton X-100) and protease inhibitors (1 mM PMSF, 10 μg/ml leupeptin, 5 μM pepstatin A, 1 mM benzamidine, 30 mM sodium fluoride, 2 mM sodium orthovanadate, and 1 μg/ml aprotinin) on ice. The supernatant was collected and assayed for protein content using the BCA protein assay (Thermoscientific). Thirty to one hundred micrograms (depending on the abundance of the signal) of protein lysate were resolved electrophoretically and transferred to Immobilon films (Millipore, Billerica, MA). Filters were blocked in 5% nonfat dried milk and 0.05% Tween and incubated with an antibody or primary antibody, dissolved in a 0.1% Triton X-100, 0.1% BSA, 1% FBS containing 0.3% Triton X-100 in PBS. Tissue auto-fluorescence was reduced by 100% Tween and immunoblotted with a primary antibody (see *Reagents*). After being washed, blots were incubated with a horseradish peroxidase-conjugated secondary antibody (Sigma, St. Louis, MO) and bands were visualized by the West Pico enhanced chemiluminescence kit (Pierce, Rockford, IL). After the membrane was stripped and blocked, the blot was incubated with an anti-actin or anti-GAPDH-specific antibody and visualized using the same methods as the primary antibody.

**Reagents.** Inhibitors were as follows: 10 μM CAY10404 (Cayman Chemical), 20 μM GW4869 (Cayman Chemical), and 100 μM AA (Cayman Chemical). Antibodies were as follows: rabbit anti-COX-2 (1:1,000; Cayman Chemical), rabbit anti-COX-1 (1:1,000; Cayman Chemical), rabbit anti-PLA2 (1:500; Cell Signaling), rabbit anti-N-SM (1:500; for Western) or (1:100; for immunofluorescence; Ab-cam), mouse anti-actin (1:1,000; Cell Signaling), mouse anti-GAPDH (Santa Cruz Biotechnology) antibody and goat anti-rabbit conjugated to horseradish peroxidase or goat anti-mouse conjugated to horseradish peroxidase (1:5,000; Sigma).

**Animals.** Six- to eight-week-old FVB male mice were obtained from Charles River Labs (Wilmington, MA) and housed at the Icahn School of Medicine Center for Comparative Medicine and Surgery. To induce a high urine flow rate, mice were subcutaneously injected with isotonic saline (volume = 20% of body wt) while the control mouse was sham injected (no fluid given). The amount of saline to safely induce a natriuretic/polyuric response was based on studies by Galla and Luke (10) who injected up to 20% of saline into rodents. The injected, volume-expanded (VE), and sham-injected, control mice were then placed in separate metabolic cages for 6 h to collect urine.

**RESULTS**

**FSS, in vitro, and high tubular flow rates, in vivo, induce PGE2 synthetic enzymes.** High-dietary NaCl (8%) ingestion in rodents induces COX-2 mRNA expression in the renal medulla (3, 34), which we speculate may, in part, be related to high urinary and tubular flow rates. Our laboratory demonstrated that a physiologic level (0.4 dyn/cm2) (14) of FSS for 2 h induces COX-2 mRNA and tended to raise COX-2 protein expression in IMCD3 cells, but this did not reach statistical significance (8). This response to FSS, we believe, was due to the fact that protein synthesis requires more time before a change in protein expression can be detected by immunoblotting. To test this, IMCD3 cells were exposed for 4 h to 0.4 dyn/cm2 of FSS or no FSS, and immunoblotting was performed on protein lysates. Immunodetectable COX-2 protein was increased in cells subject to 4 h of FSS compared with static cells (Fig. 1), suggesting FSS induces immunodetectable COX-2 protein expression.
To test whether high urine flow rates in vivo activate the intrarenal PGE2 synthetic system, specifically COX-2, mice were injected subcutaneously with isotonic saline at 20% of body weight to augment the urine flow rate and mimic in vitro FSS experiments. This maneuver suppresses serum aldosterone concentration in the absence of effects on serum vasopressin (31) and enhances urine flow rate. Mice were placed in a metabolic cage for 6 h to collect urine, killed, and then kidneys were extracted for analysis. The 6-h time point was chosen to ensure that kidneys experienced high tubular flow for at least 4 h because our in vitro data suggested this length of time was necessary to observe immunodetectable COX-2 expression. The urine volume collected from saline-injected mice (2.9 ± 0.2 ml; n = 12) was approximately sixfold greater than sham-injected control mice (0.5 ± 0.1 ml; n = 12, P < 0.05). COX-1, COX-2, and cPLA2 protein abundance were measured in cortex and medulla of saline-injected, VE, and sham-injected, control mice (Fig. 2A). In paired sets of VE and sham-injected control mice (n = 7), steady-state COX-2 protein abundance was greater by ~70% in the cortex of kidney from VE mice than from sham-injected mice (Fig. 2B; *P < 0.05). Similarly, COX-2 protein expression in medullary kidney from VE mice was ~60% greater than from uninjected control mice (Fig. 2C; *P < 0.05). However, cortical and medullary expression of COX-1 protein (Fig. 3A) was similar in VE and control mouse kidneys (n = 3; Fig. 3, B and C, respectively). The absence of significant change in COX-1 protein expression in response to VE is consistent with our previous finding that COX-1 mRNA is unchanged in FSS-exposed IMCD3 cells (8). cPLA2, a key enzyme that releases AA from cell membranes to form the substrate for COXs, is also significantly induced in both cortex (Fig. 4, A and B) and medulla (Fig. 4, C and D) of kidney from VE mice compared with uninjected control mice, suggesting intrarenal activation of the PGE2 synthetic system by high tubular flow.

**N-SM is expressed in CD and is a FSS-sensitive regulator of COX-2 protein abundance.** FSS activates N-SM activity in endothelial cells to release ceramide that stimulates COX-2 protein abundance (4, 5). We propose that a similar mechanism functions in CD cells to induce COX-2 protein abundance and augment the potential to generate PGE2. First, we verified that N-SM protein is expressed in IMCD3 cells and murine kidney by immunoblotting and immunofluorescence studies. N-SM was abundantly expressed in IMCD3 cells (Fig. 5) and in cortex and medulla of mouse kidney (Fig. 5). In addition, N-SM was localized to the CCD (Fig. 6A) in vivo. To identify principal cells (PCs), and hence the CCD, murine kidney sections were incubated with rhodamine-labeled dolichos biflorus agglutinin (red) and to label N-SM, a rabbit anti-N-SM antibody was incubated with the tissue (green). PCs (white arrows) and intercalated cells (ICs; yellow arrows) both express N-SM; however, N-SM is primarily observed in the cytoplasm of ICs while PCs express N-SM in both cytoplasm and apically (Fig. 6A).

Next, we tested whether FSS activates sphingomyelinase activity in IMCD3 cells. To this end, we first evaluated, by Western blotting, whether the supernatant (cytosolic) or pellet (nonsedimentable) fraction of IMCD3 cells expressed N-SM protein. We found that N-SM protein abundance was much greater in the supernatant than in the pellet (Fig. 7A). The sphingomyelinase activity in the supernatant of FSS-exposed and static IMCD3 cells was assayed and normalized to total protein from that fraction. We chose to expose IMCD3 cells for only 2 h of FSS, rather than 4 h, because we suspect that sphingomyelinase stimulation induces COX-2 expression, so it needs to be activated before the increase in COX-2 protein abundance. The sphingomyelinase activity was ~10-fold greater in sheared IMCD3 (32.2 ± 7.7 pmol/min/ml·μg−1 protein; *P < 0.05; Fig. 7B) than static controls (4.4 ± 1.4 pmol/min/ml·μg−1 protein). Next, we tested whether FSS induced N-SM-specific activity by exposing IMCD3 cells to FSS in the absence and presence of a specific N-SM inhibitor, GW4869 (20 μM). Approximately 50% of the shear-sensitive sphingomyelinase activity was due to N-SM (Fig. 7C).

Since FSS induced COX-2 protein and N-SM activity, shear-exposed cells were treated with GW4869 (20 μM) to examine whether inhibition of FSS-induced N-SM activity repressed FSS-induced COX-2. N-SM specific inhibition suppressed COX-2 protein abundance by >50% in sheared cells compared with that observed in untreated cells (Fig. 1), suggesting that shear-stimulated N-SM activity influences COX-2 protein abundance.

Moreover, sphingomyelinase activity generates ceramides that bind kinase suppressor of Ras (KSR) to induce Raf-1 and Ras, and thus, MAPK (4, 5, 38) to stimulate COX-2 protein abundance (27). IMCD3 cells were treated with a ceramide analog (C2-ceramide, an analog of N-acetylsphingosine) for 4 h. C2-ceramide (0–100 μM) induced COX-2 protein abundance (Fig. 8A) at concentrations of 20 and 100 μM compared with untreated controls (Fig. 8B; *P < 0.05), suggesting that the end product of sphingomyelinase activity regulates immunodetectable COX-2 expression in CD cells.

**Incubation with AA, as a method to evaluate PGE2 generating potential of epithelia.** IMCD3 cells were incubated with a high concentration of AA (100 μM), the substrate for COXs, for 1 h, and PGE2 was measured, as a surrogate of total PGE2 synthesizing potential (13, 29). IMCD3 cells were either maintained under static conditions or exposed to FSS (0.4 dyn/cm²).
for 2 h and then PGE$_2$ was measured in media in the absence or presence of AA. PGE$_2$ secretion under static conditions, in the absence of AA, was very low (1.28 ± 0.37 pg·ml$^{-1}$·µg$^{-1}$ protein) but was enhanced in static cells incubated with AA (61.7 ± 9.1 pg·ml$^{-1}$·µg$^{-1}$ protein; *P < 0.05; Fig. 9) suggesting that the cells have significant reserve to boost PGE$_2$ synthesis. Sheared cells (34.6 ± 1.2 pg·ml$^{-1}$·µg$^{-1}$ protein) also exhibited an increase in PGE$_2$ secretion after incubation with AA (92.1 ± 5.5 pg·ml$^{-1}$·µg$^{-1}$ protein; Fig. 9; *P < 0.05). Sheared cells exposed to AA released the most PGE$_2$ (SP < 0.05 vs. static with AA and shear alone) implying that cellular PGE$_2$ generating potential is increased as early as 2 h after experiencing FSS. Our earlier studies suggested no change in COX-1 mRNA, an increase in COX-2 mRNA, and a nonsignificant (P = 0.064) increase in COX-2 protein. We suspect that AA treatment of cells enhances our sensitivity to measure changes in COX protein/activity and/or the overall

Fig. 2. Volume expansion and subsequent diuresis induce renal COX-2 protein abundance. Six- to eight-week-old mice were either sham-injected or injected subcutaneously with isotonic saline at 20% of body weight. The mice were placed into a metabolic cage for 6 h and urine was collected. Kidneys were then extracted and protein lysate was generated from the cortex and medulla. A: single Western blot of renal cortex and medulla of sham- and saline-injected mice demonstrates an increase in COX-2 protein abundance. The open bar identifies that the image was spliced together from a single immunoblot. Densitometric analysis of immunoblots comparing the COX-2 expression in renal cortex (B) and medulla (C) in sham (n = 7) vs. saline-injected (n = 7) mice demonstrates an increase in COX-2 protein abundance by >60% in kidneys of the latter (*P < 0.05).

Fig. 3. Volume expansion and subsequent diuresis do not affect expression of renal COX-1 protein abundance. In identical experiments that are described in Fig. 2, COX-1 protein abundance was evaluated in the renal cortex and medulla of sham- and saline-injected mice. A: in a single Western blot, COX-1 protein abundance did not differ between renal cortex and medulla of sham- and saline-injected mice. The open bar identifies that the image was spliced together from a single immunoblot. Densitometric analysis of immunoblots comparing the COX-2 expression in renal cortex (B) and medulla (C) in sham (n = 3) vs. saline-injected (n = 3) mice demonstrates an increase in COX-2 protein abundance by >60% in kidneys of the latter (*P < 0.05).
potential of an epithelium to generate PGE2 than our immunodetection methods.

*PGE2 generation in microdissected CCDs. To evaluate whether fast urinary and tubular flow rates stimulate PGE2 synthesis in vivo, CCDs were microdissected from VE and control mice, CCDs were placed in RL for 30 min at 37°C, and PGE2 was measured in the supernatant and tubule. The urinary PGE2 concentration in saline injected mice (Fig. 10A; 445 ± 91 pg/ml; #P < 0.05) was approximately twofold greater than that measured in control mice (205 ± 14 pg/ml). As would also be expected, the PGE2 excretion over the 6 h was also significantly greater in VE (1,463 ± 180 pg of PGE2; P < 0.05) compared with sham-injected controls (112 ± 28 pg of PGE2). The concentration of PGE2 measured in the supernatant bathing microdissected CCDs (Fig. 10B; 135.8 ± 21.7 pg·ml⁻¹·mm⁻¹ CCD; #P < 0.05) and in the CCD itself (Fig. 10C; 75.0 ± 17.2 pg·ml⁻¹·mm⁻¹ CCD; #P < 0.05) was approximately twofold greater than that observed in the supernatant (65.8 ± 11.0 pg·ml⁻¹·mm⁻¹ CCD) and in CCDs (33.3 ± 10.9 pg·ml⁻¹·mm⁻¹ CCD) of control mice, respectively, suggesting that high urinary flow rates activate PGE2 synthetic machinery in vivo.

The total PGE2 generating activity of CCDs was measured by incubating CCDs in RL containing 100 μM AA and then measuring PGE2, as we did with IMCD3 cells. PGE2 concentration in the supernatant was greater in CCDs incubated in AA than untreated CCDs (Figs. 10B vs. 11), implying that CCDs from control and VE mice have greater potential to generate
PGE2. CCDs isolated from uninjected control mice secreted less PGE2 (328 ± 16 pg·ml⁻¹·min⁻¹·cell⁻¹) after exposure to 100 μM AA than VE CCDs exposed to AA (607 ± 108 pg·ml⁻¹·min⁻¹·cell⁻¹; Fig. 11; #P < 0.05), implying that the capability to synthesize PGE2 in VE mice is approximately twofold greater than control mice. Incubating CCDs of VE mice with a COX-2-specific inhibitor (CAY10404) and AA suppressed the PGE2 to 336 ± 34 pg·ml⁻¹·min⁻¹·cell⁻¹, which is not different than CCDs isolated from sham-injected mice. This suggested that the AA-mediated increase in PGE2 from CCDs isolated from VE mice was largely due to an increase in COX-2 activity/protein.

DISCUSSION

Growing evidence points to the critical role that hydrodynamic forces play in transepithelial transport, signaling and morphology of the kidney (6–8, 18–20, 26). This study seeks to contribute to this literature by showing that high tubular flow rates in vivo, as follows isotonic volume expansion, induce CD expression of COX activity and enhance PGE2 release which we propose facilitates Na excretion. In vitro studies of an IMCD3 cell culture model revealed that FSS-induced COX-2 protein expression is regulated by N-SM activity, a mechanism known to regulate flow-mediated COX-2 expression in endothelia (4, 5, 27).

We demonstrate that high tubular flow in vivo produced by isotonic extracellular volume expansion 1) enhances PGE2 secretion into urine, 2) induces PGE2 secretion by isolated CCDs, 3) stimulates cortical and medullary COX-2 protein abundance, and 4) boosts total PGE2 generating activity, which is largely COX-2 activity in CCDs. FSS, a hydrodynamic force generated by fast tubular flow rates applied to our in vitro CD cell culture model, stimulates COX-2 protein expression through N-SM-dependent activity. Moreover, N-SM, the FSS-sensitive sphingomyelinase isoform expressed in IMCD3 cells, is also expressed in murine CD cells (PCs and ICs), suggesting that this shear-sensitive pathway may exist in a native kidney; however, further studies are required to confirm this mechanism in vivo.

Prior studies by others showed that high-Na diets increased urinary PGE2 excretion and medullary COX-2 expression, as we did; however, these investigators identified enhanced im-
munodetectable COX-2 protein in interstitial cells (3, 36), not in CDs. In fact, immunodetectable COX-2 was not identified in CDs in either control or high-Na diet animals (36). Our studies suggest that, in both microdissected CCDs and cultured CD cells, flow and shear augment total PGE2 generating activity and, specifically, COX-2 activity/protein. In our murine model, an increase in tubular flow induces an approximately twofold rise in urinary PGE2 concentration and CCD-derived PGE2 within 6 h, suggesting that urinary PGE2 reflects CD metabolism of AA. Moreover, renal excretion of PGE2, as measured in
the urine, is >10-fold greater in polyuric mice compared with control mice. We speculate the difference in COX-2 activity and localization between the studies by Ye et al. (36) and our current study may reflect differences in the species examined (rat vs. mouse), 2) immunodetection vs. enzymatic activity to assess COX expression, 3) Na ingestion vs. isotonic saline injection, and 4) chronic (3 days) vs. acute (6 h) study (36).

The data fit well within our conceptual framework that tubular flow and FSS rapidly 1) activate cPLA2 through changes in intracellular calcium concentration and phosphorylation to augment AA generation (8) and 2) induce longer-term adaptive changes in COX protein abundance or activity to boost the capacity to convert AA to its intermediate endoperoxides and, hence, PGE2. This model emerged from our previous work demonstrating that FSS rapidly induces cPLA2 phosphorylation and PGE2 generation in IMCD3 cells, while COX-2 protein content was not statistically greater than that observed in static control cells (8). In this paper, 4 h of FSS stimulated N-SM-dependent COX-2 protein expression in IMCD3 cells. Moreover, PGE2 secreted by microdissected CCDs was greater in polyuric than in control kidneys and, importantly, incubation of CCDs with AA was able to further enhance PGE2 generation, principally through a COX-2-dependent pathway. This suggested to us that acute flow-mediated PGE2 release requires generation of AA, principally by cPLA2 activation (8). On the other hand, chronic high tubular flow, which accompanies high-Na diets, stimulates COX-2 activity and/or protein to ensure that the total COX activity is sufficient to rapidly process AA to its endoperoxide intermediates so that PGE2 can be abundantly released and effectuate Na excretion.

Pathologic renal conditions are also associated with high urine/tubular flow rates and increased levels of urinary PGE2 excretion and renal PGE2 generation. Before the molecular mechanism underlying Bartter’s syndrome was identified, it was already established that urinary PGE2 was elevated in Bartter’s syndrome and that inhibition of renal PGE2 synthesis with indomethacin (COX-1 and COX-2 inhibitor) restored Na and K balance (2). Further studies implicated that COX-2 was the principal COX isoform contributing to high levels of urinary PGE2 and that inhibition of COX-2, specifically, suppressed renal PGE2 release and polyuria (21–23). In addition, autosomal dominant polycystic kidney disease often presents with polyuria and an inability to maximally concentrate the urine (24). Kidneys of rodent and murine models of PKD show...
elevated cPLA2 protein and total COX activity compared with control mice, and COX-2-specific inhibition suppresses cystogenesis, fibrosis, and macrophage infiltration (1, 25, 30). We speculate that high rates of urine and tubular flow contribute to enhanced renal COX-2 activity and PGE2 generation which, depending on the context of PGE2 release, plays a physiologic (Na and K regulation) or pathophysiologic (proliferation and cystogenesis) role in the kidney (15).

It should be noted that our study does have limitations. Specifically, utilizing our IMCD3 model, we were able to demonstrate the FSS-induced N-SM activity regulates COX-2 protein expression; however, flow activation of N-SM pathway in the native tubule/kidney was not demonstrated, although CCD expression of N-SM in kidney was observed. In regards to the murine model, isotonic saline injection is a crude method to induce high tubular flow rates since injection of saline affects the neurohormonal axis, such as suppressing aldosterone, renin, and angiotensin II. Because the renin-angiotensin-aldosterone axis is suppressed and vasopressin is unaffected by isotonic saline (31), we suspect that change in tubular flow to the murine model, isotonic saline injection is a crude method to induce high tubular flow rates since injection of saline affects the neurohormonal axis, such as suppressing aldosterone, renin, and angiotensin II. Because the renin-angiotensin-aldosterone axis is suppressed and vasopressin is unaffected by isotonic saline (31), we suspect that change in tubular flow to be the predominant mechanism of action on the tubule. Atrial natriuretic peptide (ANP), a small peptide hormone that enables the predominant mechanism of action on the tubule. Atrial natriuretic peptide (ANP), a small peptide hormone that enhances Na excretion at the CD, is likely elevated with volume expansion; however, little evidence points to ANP regulating CD expression of PGE2.

In sum, our studies are the first to demonstrate that FSS regulates COX-2 protein expression in a CD cell model through a sphingomyelinase-dependent mechanism. In addition, we demonstrate that CCDs exposed to high tubular flows secrete greater amounts of PGE2 which, likely, effectuate enhanced renal Na excretion. Finally, tubular flow augments CCD COX activity through stimulation of COX-2 protein or activity that has not been demonstrated in other murine models.

ACKNOWLEDGMENTS

We gratefully acknowledge the informative discussions with Lisa Satlin and the support of her lab members (Carlos Schrek and Yuehan Zhou) in teaching us the microdissection technique. Microscopic analysis was performed in the Microscopy Shared Resource Facility.

GRANTS

This work was supported by the Department of Veterans Affairs Merit Review 1101BX000388 (R. Rohatgi) and the Bronx Veterans Medical Research Foundation.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: Y.L., D.F., and R.C.-G. performed experiments; Y.L. and R.C.-G. analyzed data; Y.L. and D.F. prepared figures; R.R. conception and design of research; R.R. interpreted results of experiments; R.R. drafted manuscript; R.R. edited and revised manuscript; R.R. approved final version of manuscript.

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