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8-iso-prostaglandin-F2α stimulates chloride transport in thick ascending limbs: role of cAMP and protein kinase A

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[1]*Hypertension and Vascular Research Division, Department of Internal Medicine, Henry Ford Hospital, Detroit, Michigan;* [2]*Department of Renal Physiology, J. Robert Cade Foundation CONICET, Cordoba;* [3]*Department of Renal Physiology and Hypertension, Mons. Carlos V. Cruvellier Foundation, San Juan, Argentina; and* [4]*Department of Physiology and Biophysics and Division of Nephrology, Department of Medicine, University of Mississippi Medical Center, Jackson, Mississippi*

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Cabral PD, Silva GB, Baigorria ST, Juncos LA, Juncos LI, García NH. 8-iso-prostaglandin-F2α stimulates chloride transport in thick ascending limbs: role of cAMP and protein kinase A. *Am J Physiol Renal Physiol* 299: F1396–F1400, 2010. First published September 22, 2010; doi:10.1152/ajprenal.00225.2010.—Salt reabsorption by the loop of Henle controls NaCl handling and blood pressure regulation. Increased oxidative stress stimulates NaCl transport in one specific segment of the loop of Henle called the thick ascending limb (TAL). The isoprostane 8-iso-prostaglandin-F2α (8-iso-PGF2α) is one of the most abundant nonenzymatic lipid oxidation products and has been implicated in the development of hypertension. However, it is not known whether 8-iso-PGF2α regulates transport or the mechanisms involved. Because protein kinase A (PKA) stimulates NaCl transport in several nephron segments, we hypothesized that 8-iso-PGF2α increases NaCl transport in the cortical TAL (cTAL) via a PKA-dependent mechanism. We examined the effect of luminal 8-iso-PGF2α on NaCl transport by measuring chloride absorption (J_{Cl}) in isolated microperfused cTALs. Adding 8-iso-PGF2α to the lumen increased J_{Cl} by 54% (from 288.7 ± 30.6 to 446.5 ± 44.3 pmol·min^{-1}·mm^{-1}; $P < 0.01$), while adding it to the bath enhanced J_{Cl} by 35% (from 236.3 ± 35.3 to 319.2 ± 39.8 pmol·min^{-1}·mm^{-1}; $P < 0.05$). This stimulation was blocked by Na-K-2Cl cotransporter inhibition. Next, we tested the role of cAMP. Basal cAMP in the cTAL was 18.6 ± 1.6 fmol·min^{-1}·mm^{-1}, and 8-iso-PGF2α raised it to 35.1 ± 1.4 fmol·min^{-1}·mm^{-1}, an increase of 94% ($P < 0.01$). Because cAMP stimulates PKA, we measured J_{Cl} using the PKA-selective inhibitor H89. In the presence of H89 (10 μM), 8-iso-PGF2α failed to increase transport regardless of whether it was added to the lumen (216.1 ± 16.7 vs. 209.7 ± 23.8 pmol·min^{-1}·mm^{-1}; NS) or the bath (150.4 ± 32.9 vs. 127.1 ± 28.6 pmol·min^{-1}·mm^{-1}; NS). We concluded that 8-iso-PGF2α stimulates cAMP and increases Cl transport in cTALs via a PKA-dependent mechanism.

salt reabsorption; cortico-medullary osmotic gradient

THE THICK ASCENDING LIMB of the loop of Henle reabsorbs 20–30% of filtered NaCl and thus is critical to salt regulation and fluid homeostasis (12). This nephron segment is impermeable to water and regulates the cortico-medullary osmotic gradient, which determines how much water is absorbed by the collecting ducts (6). Several factors including increased NaCl (32), ANG II-dependent hypertension (31), and increased luminal flow (14) increase free radical production in this segment.

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Free radicals rapidly attack lipids, forming several types of peroxidation products (27). One that is formed in abundance both in vitro and in vivo is 8-iso-prostaglandin-F2α (8-iso-PGF2α) (26), a member of the isoprostane family: these are prostaglandin-like compounds produced by nonenzymatic free radical peroxidation of arachidonic acid (20).

8-iso-PGF2α and its derivatives have the potential to act as antidiuretics by affecting renal hemodynamics or tubular transport. In the kidney, 8-iso-PGF2α induces vasoconstriction (4) and this could reduce renal blood flow and glomerular filtration rate. While these mechanisms could lower Na and water excretion by themselves, it is probable that 8-iso-PGF2α also has a direct effect on tubular transport.

Increased concentrations of 8-iso-PGF2α and other isoprostanes have been observed in both the urine (28) and plasma (13, 25) of hypertensive subjects (13, 18, 19, 25, 28), suggesting that 8-iso-PGF2α may affect renal tubular function from both the luminal and basolateral side.

In other types of cells, increased 8-iso-PGF2α has been linked to cyclic adenosine monophosphate (cAMP)-dependent pathways (16). In the thick ascending limb, increased cAMP heightens NaCl transport (22); however, the effect of 8-iso-PGF2α on cAMP in the cortical thick ascending limb is unclear. It is well-documented that cAMP binds to and activates protein kinase A (34), causing NaCl transport to increase in several nephron segments including the thick ascending limb (1, 5). However, it is unknown whether protein kinase A mediates the actions of 8-iso-PGF2α in the cortical thick ascending limb. Therefore, we tested the hypothesis that 8-iso-PGF2α increases transport in cortical thick ascending limbs via a mechanism mediated by protein kinase A.

METHODS

Animals. Male New Zealand White rabbits (700–1,200 g) were housed for 3–7 days before the study. All experiments were approved by the Institutional Animal Care and Use Committee of the J. Robert Cade Foundation before performing any procedures on animals. All animals were housed and handled in accord with the Public Health Service Policy on Humane Care and Use of Laboratory Animals.

Preparation and dissection of cortical thick ascending limbs. Animals were anesthetized with Na thiopental (30 mg/kg iv) and xylazine (20 mg/kg im) plus ketamine (50 mg/kg im) and the abdominal cavity was opened. The kidney was bathed in ice-cold saline, removed, and cut into coronal slices along the longitudinal axis. The slices were placed in oxygenated physiological saline in a temperature-controlled chamber at 4°C. Cortical thick ascending limbs were dissected from the medullary rays under a stereomicroscope as described previously (11, 33).

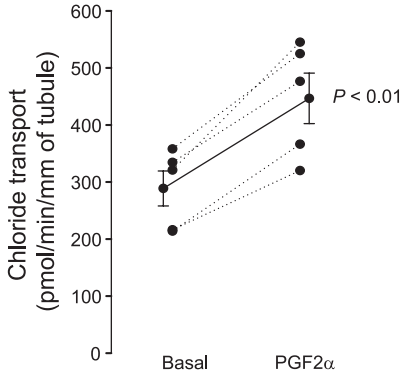

Fig. 1. Effect of luminal 8-iso-prostaglandin-F2α (PGF2α; 100 μM) on chloride absorption (J_{Cl}; $n = 5$).

Perfusion of thick ascending limb tubules. Cortical thick ascending limbs were transferred to a temperature-regulated chamber and perfused between concentric glass pipettes at 37°C as described previously (33). The composition of the basolateral bath and perfusate was (in mM) 114 NaCl, 25 $NaHCO_3$, 2.5 NaH_2PO_4, 4 KCl, 1.2 $MgSO_4$, 6 alanine, 1 trisodium citrate, 5.5 glucose, and 2 calcium dilactate. Solutions were bubbled with 5% CO_2-95% O_2 before and during the experiment. The pH of the solutions was 7.4 and osmolality 290 ± 3 mosmol/kgH_2O as measured by freezing-point depression. The basolateral bath was exchanged at a rate of 0.5 ml/min. A total of five to eight tubules were analyzed during each protocol. Time control experiments were conducted to ensure stability of tubular transport.

Measurement of Cl absorption. Chloride absorption was measured as described previously (10, 33). Thick ascending limbs were perfused and equilibrated for 20 min and basal Cl absorption was calculated two or three times. Tested compounds, including 8-iso-PGF2α, were either added to or removed from the lumen or bath as indicated. After a 20-min equilibration period, tubular fluid was again collected two or three times. Chloride concentrations in the perfusate and collected fluid were measured by microfluorometry. Because water is not reabsorbed by the thick ascending limb, chloride absorption (J_{Cl}) was calculated as follows: $J_{Cl} = CR(C_{0Cl} - C_{1Cl})$, where CR is collection rate normalized per tubule length, C_{0Cl} is chloride concentration in the perfusion solution, and C_{1Cl} is chloride concentration in the collected fluid.

cAMP measurements. Tubules were incubated in a 95-μl perfusion solution containing 1 mM 3-isobutyl-1-methylxanthine at 37°C for 10 min before adding a 5-μl medium containing the different inhibitors or hormones. After 30 min, the reaction was stopped with methanol (100 μl) and cAMP was determined with an enzyme-immunoassay kit (EIA, Cayman). On the day of the assay, samples were centrifuged, the supernatant was transferred to another tube, dried in a Savant, and the pellet was reconstituted in 110 μl Na acetate buffer. cAMP standards were treated the same way. The results were expressed as femtomoles per minute per millimeter of tubule length.

Statistics. All data were analyzed by the Department of Statistics and Epidemiology of the J. Robert Cade Foundation. Experimental results were expressed as means ± SE. Data were evaluated using a paired Student's *t*-test and unpaired *t*-test, taking $P < 0.05$ as significant.

RESULTS

8-iso-PGF2α increased J_{Cl} in cortical thick ascending limbs via either the luminal or basolateral membrane. Under basal conditions, J_{Cl} was 288.7 ± 30.6 pmol·min^{-1}·mm^{-1}. After 8-iso-PGF2α (100 μM) was added to the lumen, it rose to 446.5 ± 44.3 pmol·min^{-1}·mm^{-1}, an increase of 54% ($n = 5$; $P < 0.01$; Fig. 1). Lower concentrations (0.01 and 1 μM) had no significant effect on J_{Cl} (5.4 ± 7.9 and 10.1 ± 7.5%, respectively). These data indicate that luminal stimulation with 8-iso-PGF2α enhances Cl transport in cortical thick ascending limbs.

Since the basolateral side of the cortical thick ascending limb is in direct contact with capillaries such as the vasa recta, we investigated the effect of 8-iso-PGF2α added to the basolateral side. Basal J_{Cl} was 236.3 ± 35.3 pmol·min^{-1}·mm^{-1}, and after adding 8-iso-PGF2α to the basolateral side, it rose to 319.2 ± 39.8 pmol·min^{-1}·mm^{-1}, an increase of 35% ($n = 5$; $P < 0.05$; Fig. 2). In time control experiments, J_{Cl} remained constant throughout the experimental period (from 320.7 ± 51.8 to 313.0 ± 76.4 pmol·min^{-1}·mm^{-1}; NS vs. basal; $n = 8$). Taken together, these data indicate that 8-iso-PGF2α increases cortical thick ascending limb Cl reabsorption by acting on either the luminal or basolateral membrane.

Na-K-2Cl cotransport inhibitor furosemide blocked 8-iso-PGF2α-stimulated J_{Cl} in cortical thick ascending limbs. Most NaCl reabsorption by the thick ascending limb is due to Na-K-2Cl cotransport (12). Therefore, we tested whether furosemide inhibits 8-iso-PGF2α-stimulated J_{Cl}. In control experiments, basal J_{Cl} was 227.7 ± 17.6 pmol·min^{-1}·mm^{-1}, and after adding furosemide (100 μM) to the lumen, it dropped to 42.2 ± 13.3 pmol·min^{-1}·mm^{-1} ($P < 0.01$; $n = 5$). When we removed the furosemide from the lumen, J_{Cl} rose to 259.7 ± 26.6 pmol·min^{-1}·mm^{-1}, indicating that furosemide at the concentration we used had no toxic effects on our preparations.

Next, we tested the effect of furosemide on 8-iso-PGF2α-stimulated J_{Cl}. In the absence of furosemide, but in the presence of 8-iso-PGF2α added to the luminal side, J_{Cl} was 314.2 ± 36.3 pmol·min^{-1}·mm^{-1}; and when we added furosemide to the lumen, it fell to 86.4 ± 29.6 pmol·min^{-1}·mm^{-1}, representing a 73% blockade ($n = 5$; $P < 0.01$ vs. 8-iso-PGF2α; Fig. 3). We concluded that 8-iso-PGF2α-stimulated Cl transport can be abolished by blocking Na-K-2Cl cotransport.

Then, we investigated the role of furosemide in blocking the effect of 8-iso-PGF2α added to the basolateral membrane. In the absence of furosemide, but in the presence of 8-iso-PGF2α added to the bath, J_{Cl} was 326.5 ± 85.3 pmol·min^{-1}·mm^{-1}; after adding furosemide to the lumen, it fell to 107.5 ± 27.6 pmol·min^{-1}·mm^{-1}, representing a 67% blockade ($n = 5$; $P < 0.05$ vs. 8-iso-PGF2α; Fig. 4). Taken together, these data indicate that 8-iso-PGF2α-stimulated J_{Cl} in cortical thick ascending limbs can be diminished by blocking Na-K-2Cl cotransport.

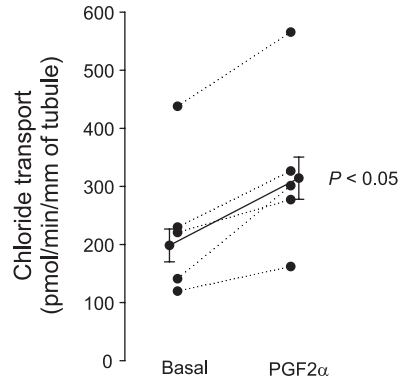

Fig. 2. Effect of basolateral PGF2α on J_{Cl} ($n = 5$).

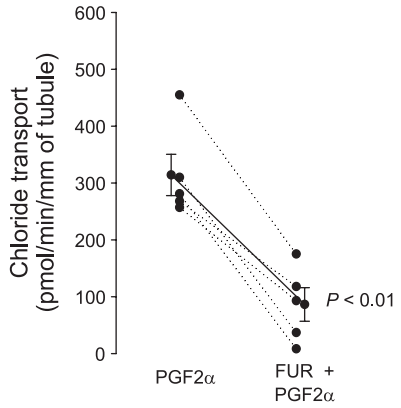

Fig. 3. Effect of furosemide (FUR; 100 μM) on luminal PGF2α-stimulated J_{Cl} ($n = 5$).

8-iso-PGF2α increased J_{Cl} in cortical thick ascending limbs via a cAMP/protein kinase A-dependent mechanism. In several nephron segments, including the thick ascending limb, cAMP increases NaCl transport (22). Therefore, we tested whether 8-iso-PGF2α increases cAMP in the cortical thick ascending limb. We found that vehicle stimulated cAMP to 18.6 ± 1.6 fmol·min^{-1}·mm^{-1}, while adding 8-iso-PGF2α increased it to 35.1 ± 1.4 fmol·min^{-1}·mm^{-1}, a 94% increase ($n = 6$; $P < 0.01$; Fig. 5). These data indicate that 8-iso-PGF2α increases cAMP in cortical thick ascending limbs.

In the thick ascending limb, cAMP stimulates protein kinase A activity and subsequently increases Na-K-2Cl cotransporter insertion in the luminal membrane of the thick ascending limb (22), therefore we studied the effect of 8-iso-PGF2α on J_{Cl} in the presence of the protein kinase A-selective inhibitor H89. J_{Cl} was 216.1 ± 16.7 pmol·min^{-1}·mm^{-1} of tubule in the presence of H89 (10 μM) and did not change significantly after we added 8-iso-PGF2α to the luminal side (209.7 ± 23.8 pmol·min^{-1}·mm^{-1}; $n = 6$; NS vs. H89; Fig. 6). These data indicate that 8-iso-PGF2α added to the luminal side increases J_{Cl} in cortical thick ascending limbs primarily via protein kinase A.

In the thick ascending limb, reactive oxygen species or its derivatives activate different signaling pathways depending on whether they act on the luminal or basolateral membrane (30). Therefore, we tested whether protein kinase A mediates the effect of 8-iso-PGF2α when added to the basolateral side. In

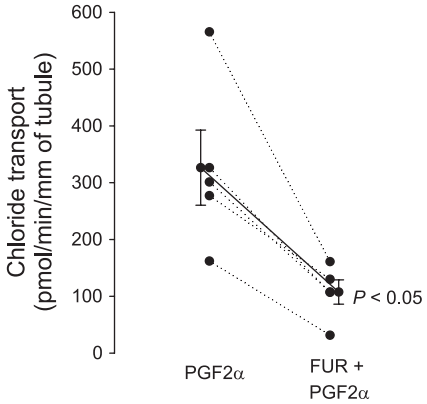

Fig. 4. Effect of FUR (100 μM) on basolateral PGF2α-stimulated J_{Cl} ($n = 5$).

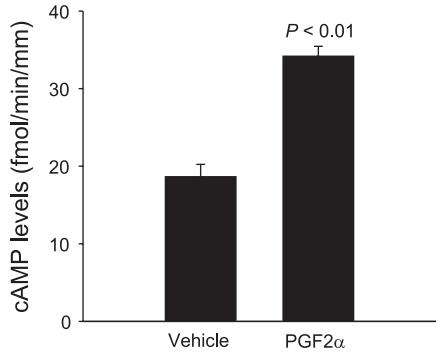

Fig. 5. Effect of PGF2α on cAMP levels ($n = 6$).

the presence of H89, J_{Cl} was 150.4 ± 32.9 pmol·min^{-1}·mm^{-1}; after adding 8-iso-PGF2α to the bath, it was 127.1 ± 28.6 pmol·min^{-1}·mm^{-1}, which is not a significant decrease ($n = 6$; NS vs. basal; Fig. 7). Taken together, these data indicate that both luminal and basolateral sources of 8-iso-PGF2α increase Cl transport in cortical thick ascending limbs primarily via a protein kinase A-dependent mechanism.

DISCUSSION

8-iso-PGF2α is a product of nonenzymatic lipid oxidation, which increases in hypertension (13, 18, 19, 25, 28); however, its contribution to cortical thick ascending limb transport is not known. We found that 8-iso-PGF2α increased Cl absorption by the cortical thick ascending limb when added to either lumen or bath and this increase was blocked by furosemide. We also found that the increased transport stimulated by 8-iso-PGF2α correlated with increased production of cAMP, a second messenger that increases NaCl transport in the thick ascending limb (22). Finally, we found that 8-iso-PGF2α-stimulated Cl transport could be inhibited by blocking protein kinase A. To our knowledge, this is the first reported documentation that 8-iso-PGF2α increases Cl transport in the cortical thick ascending limb of the loop of Henle via protein kinase A.

Several in vivo studies showed that 8-iso-PGF2α levels are increased in plasma and urine in patients with hypertension (13, 18, 19, 25, 28). This raises the question of whether 8-iso-PGF2α contributes to the development of hypertension.

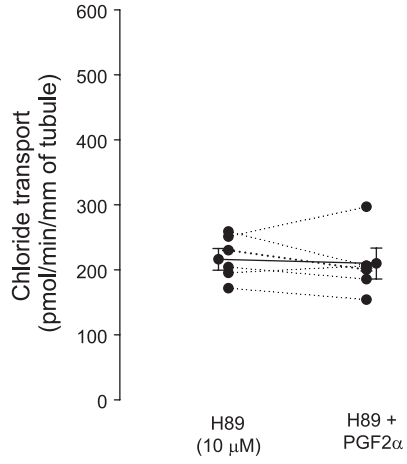

Fig. 6. Effect of PGF2α applied to the luminal side in the presence of a protein kinase A inhibitor (H89; 10 μM; $n = 6$).

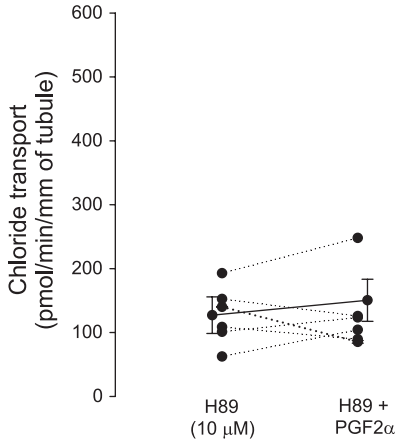

Fig. 7. Effect of PGF2α applied to the basolateral side in the presence of H89 (10 μM; $n = 6$).

It is well-documented that NaCl retention in this segment certainly does and 8-iso-PGF2α increases NaCl retention (19). Although studies showed that the antidiuretic effect of 8-iso-PGF2α may be due to changes in renal hemodynamics, the present study demonstrates that 8-iso-PGF2α also has a direct effect on epithelial transport in cortical thick ascending limbs.

Because high plasma (13, 25) and urine (28) levels of 8-iso-PGF2α are present during hypertension, we studied the effects of 8-iso-PGF2α added to the tubular lumen or bath. In the renal epithelium, reactive oxygen species or its derivatives have different effects on transporters activity when stimulated on the luminal or basolateral membrane. In the medullary thick ascending limb, luminal superoxide stimulation increases luminal Na/H exchanger activity, whereas basolateral superoxide stimulation inhibits basolateral Na/H exchanger activity (15). Here, we demonstrate that this is apparently not the case for 8-iso-PGF2α, since Cl transport was stimulated from both luminal and basolateral sides. The effect of 8-iso-PGF2α on Cl transport resembles that of hormones such as ANG II (24), dopamine (9), or autacoids such as prostaglandins (8), which are also active via both the luminal and basolateral membrane.

During several pathological conditions such as hypertension, chronic kidney disease, and cirrhosis, levels of 8-iso-PGF2α in plasma and urine are increased. Since 8-iso-PGF2α enhances Cl reabsorption in this segment and could contribute to NaCl retention, we questioned whether a powerful diuretic such as furosemide could ameliorate the effect of 8-iso-PGF2α on Cl transport. Although 8-iso-PGF2α increased NaCl transport, this effect can be easily reversed with a diuretic such as furosemide.

Our data demonstrate that 8-iso-PGF2α stimulates Cl absorption by cortical thick ascending limbs and is also associated with increased levels of cAMP. In several nephron segments, cAMP stimulates transport (17, 29). In the thick ascending limb, NaCl is transported mainly through Na-K-2Cl cotransport via a mechanism regulated by cAMP. Ortiz (22) showed that in the thick ascending limb increased cAMP stimulates transport by enhancing Na-K-2Cl cotransporter fusion to the apical membrane. Therefore, it is possible that 8-iso-PGF2α increases Cl transport in cortical thick ascending limbs by enhancing the Na-K-2Cl cotransporter trafficking to the apical membrane.

cAMP exerts many of its effects via activation of protein kinase A, also known as cAMP-dependent protein kinase (34). We found that protein kinase A mediated the increase in Cl transport caused by 8-iso-PGF2α in cortical thick ascending limbs. This supports in vitro studies showing that protein kinase A activation increased Na uptake in oocytes from *Xenopus laevis* transfected with the Na-K-2Cl cotransporter (23). Blocking protein kinase A with H89 inhibited Na-K-2Cl cotransport in medullary thick ascending limb suspensions (1), while increased protein kinase A activity stimulated the Na-K-2Cl cotransporter trafficking and fusion to the apical membrane (7), a process that increases NaCl transport in the thick ascending limb (22). Although we showed that the increase in Cl transport caused by 8-iso-PGF2α depends on protein kinase A, we still need to clarify exactly how this kinase enhances Na-K-2Cl cotransporter activity.

Reactive oxygen species have different effects on kinases depending on whether they act on intracellular or extracellular compartments (30). We tested whether protein kinase A mediates the effect of 8-iso-PGF2α on transport when applied from the luminal or basolateral side but found that it made no difference. Unlike other reactive oxygen species (30), the compartmentalized signaling stimulated by 8-iso-PGF2α has the same characteristics whether accessed from the luminal or basolateral membrane.

Increased renal generation or excretion of 8-iso-PGF2α has been reported in several models of hypertension, including spontaneously hypertensive rats (28), ANG II-dependent (21) and salt-sensitive (37) hypertension. Schnackenberg and Wilcox (28) showed that agents that reduce oxidative stress decrease urinary excretion of 8-iso-PGF2α in spontaneously hypertensive animals and lower blood pressure. Therefore, it is possible that increased NaCl retention by the thick ascending limb induced by 8-iso-PGF2α is a contributing factor in hypertension.

We are aware that levels of 8-iso-PGF2α and other isoprostanes in urine and plasma are much lower (4). One potential concern of our study is the relatively high concentration of 8-iso-PGF2α concentration used. However, we found that 8-iso-PGF2α at lower concentrations had little or no effect on J_{Cl}, consistent with previous studies showing that high levels of 8-iso-PGF2α were needed to elicit biological effects in the kidney. For instance, Welch (35) reported that 100 μM 8-iso-PGF2α enhanced tubuloglomerular feedback by 20%.

Up to date, the 8-iso-PGF2α levels in the renal interstitial space surrounding cortical thick ascending limbs are unknown. Since 8-iso-PGF2α is formed by lipid peroxidation of plasma membrane-derived arachidonic acid, it could spill over into the surrounding interstitial space and after that, to plasma and urine. Therefore, 8-iso-PGF2α may reach high concentrations at considerable short periods of time after being released, while it could be diluted thereafter. Consequently, it is quite plausible that the local levels of 8-iso-PGF2α may be much higher than urine and plasma.

Most of the renal effects of 8-iso-PGF2α have been attributed to activation of thromboxane receptors (35). These receptors are expressed in renal epithelial cells (2, 3, 35) and specifically in thick ascending limb cells (3). In addition, others found that orthograde loop perfusion with thromboxane mimetics increased Cl transport in this segment (36). Therefore,

the effects we observed are most likely the result of 8-iso-PGF2α-mediated activation of the thromboxane receptor.

In conclusion, we found that both luminal and basolateral administration of 8-iso-PGF2α enhanced Cl transport in cortical thick ascending limbs via a protein kinase A-dependent mechanism and this process was suppressed by blocking the Na-K-2Cl cotransporter. We also found a clear link between the increase in Cl transport caused by 8-iso-PGF2α and higher cAMP levels. Potentially, the heightened NaCl retention caused by 8-iso-PGF2α may contribute to the pathogenesis of hypertension.

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DISCLOSURES

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

REFERENCES

1. **Amlal H, LeGoff C, Vernimmen C, Paillard M, Bichara M.** Na^+-K^+(NH_4^+)-$2Cl^-$ cotransport in medullary thick ascending limb: control by PKA, PKC, and 20-HETE. *Am J Physiol Cell Physiol* 271: C455–C463, 1996.
2. **Araujo M, Welch WJ.** Cyclooxygenase 2 inhibition suppresses tubuloglomerular feedback: roles of thromboxane receptors and nitric oxide. *Am J Physiol Renal Physiol* 296: F790–F794, 2009.
3. **Asano K, Taniguchi S, Nakao A, Maruyama T, Watanabe T, Kurokawa K.** Distribution of thromboxane A2 receptor mRNA along the mouse nephron segments. *Biochem Biophys Res Commun* 226: 613–617, 1996.
4. **Badr KF, Abi-Antoun TE.** Isoprostanes and the kidney. *Antioxid Redox Signal* 7: 236–243, 2005.
5. **Bae HR, Verkman AS.** Protein kinase A regulates chloride conductance in endocytic vesicles from proximal tubule. *Nature* 348: 637–639, 1990.
6. **Burg MB.** Thick ascending limb of Henle's loop. *Kidney Int* 22: 454–464, 1982.
7. **Caceres PS, Ares GR, Ortiz PA.** cAMP stimulates apical exocytosis of the renal Na^+-K^+-$2Cl^-$ cotransporter NKCC2 in the thick ascending limb: role of protein kinase A. *J Biol Chem* 284: 24965–24971, 2009.
8. **Fine LG, Trizna W.** Influence of prostaglandins on sodium transport of isolated medullary nephron segments. *Am J Physiol Renal Fluid Electrolyte Physiol* 232: F383–F390, 1977.
9. **Garcia NH, Berndt TJ, Tyce GM, Knox FG.** Chronic oral L-DOPA increases dopamine and decreases serotonin excretions. *Am J Physiol Regul Integr Comp Physiol* 277: R1476–R1480, 1999.
10. **Garcia NH, Plato CF, Garvin JL.** Fluorescent determination of chloride in nanoliter samples. *Kidney Int* 55: 321–325, 1999.
11. **Garcia NH, Plato CF, Stoos BA, Garvin JL.** Nitric oxide-induced inhibition of transport by thick ascending limbs from Dahl salt-sensitive rats. *Hypertension* 34: 508–513, 1999.
12. **Greger R.** Ion transport mechanisms in thick ascending limb of Henle's loop of mammalian nephron. *Physiol Rev* 65: 760–797, 1985.
13. **Haas JA, Krier JD, Bolterman RJ, Juncos LA, Romero JC.** Low-dose angiotensin II increases free isoprostane levels in plasma. *Hypertension* 34: 983–986, 1999.
14. **Hong NJ, Silva GB, Garvin JL.** PKC-α mediates flow-stimulated superoxide production in thick ascending limbs. *Am J Physiol Renal Physiol* 298: F885–F891, 2010.
15. **Juncos R, Hong NJ, Garvin JL.** Differential effects of superoxide on luminal and basolateral Na^+/H^+ exchange in the thick ascending limb. *Am J Physiol Regul Integr Comp Physiol* 290: R79–R83, 2006.
16. **Khasawneh FT, Huang JS, Mir F, Srinivasan S, Tiruppathi C, Le Breton GC.** Characterization of isoprostane signaling: evidence for a unique coordination profile of 8-iso-PGF(2alpha) with the thromboxane A(2) receptor, and activation of a separate cAMP-dependent inhibitory pathway in human platelets. *Biochem Pharmacol* 75: 2301–2315, 2008.
17. **Laycock JF, Hubbard JI, Schwartz JH, Stanton BA, Valtin H.** The cAMP agonist Sp-cAMPS stimulates osmotic water transport across rat inner medullary collecting duct cells. *Ann NY Acad Sci* 689: 606–608, 1993.
18. **Lerman LO, Nath KA, Rodriguez-Porcel M, Krier JD, Schwartz RS, Napoli C, Romero JC.** Increased oxidative stress in experimental renovascular hypertension. *Hypertension* 37: 541–546, 2001.
19. **Meng S, Roberts LJ, Cason GW, Curry TS, Manning RD Jr.** Superoxide dismutase and oxidative stress in Dahl salt-sensitive and -resistant rats. *Am J Physiol Regul Integr Comp Physiol* 283: R732–R738, 2002.
20. **Morrow JD, Minton TA, Mukundan CR, Campbell MD, Zackert WE, Daniel VC, Badr KF, Blair IA, Roberts LJ.** Free radical-induced generation of isoprostanes in vivo. Evidence for the formation of D-ring and E-ring isoprostanes. *J Biol Chem* 269: 4317–4326, 1994.
21. **Ortiz MC, Manriquez MC, Romero JC, Juncos LA.** Antioxidants block angiotensin II-induced increases in blood pressure and endothelin. *Hypertension* 38: 655–659, 2001.
22. **Ortiz PA.** cAMP increases surface expression of NKCC2 in rat thick ascending limbs: role of VAMP. *Am J Physiol Renal Physiol* 290: F608–F616, 2006.
23. **Plata C, Mount DB, Rubio V, Hebert SC, Gamba G.** Isoforms of the Na-K-2Cl cotransporter in murine TAL II. Functional characterization and activation by cAMP. *Am J Physiol Renal Physiol* 276: F359–F366, 1999.
24. **Quan A, Baum M.** Endogenous production of angiotensin II modulates rat proximal tubule transport. *J Clin Invest* 97: 2878–2882, 1996.
25. **Reckelhoff JF, Zhang H, Srivastava K, Roberts LJ, Morrow JD, Romero JC.** Subpressor doses of angiotensin II increase plasma F(2)-isoprostanes in rats. *Hypertension* 35: 476–479, 2000.
26. **Roberts LJ, Moore KP, Zackert WE, Oates JA, Morrow JD.** Identification of the major urinary metabolite of the F2-isoprostane 8-iso-prostaglandin F2alpha in humans. *J Biol Chem* 271: 20617–20620, 1996.
27. **Roberts LJ, Morrow JD.** The generation and actions of isoprostanes. *Biochim Biophys Acta* 1345: 121–135, 1997.
28. **Schnackenberg CG, Wilcox CS.** Two-week administration of tempol attenuates both hypertension and renal excretion of 8-Iso prostaglandin $F_{2\alpha}$. *Hypertension* 33: 424–428, 1999.
29. **Shimizu T, Yoshitomi K, Nakamura M, Imai M.** Effects of PTH, calcitonin, and cAMP on calcium transport in rabbit distal nephron segments. *Am J Physiol Renal Fluid Electrolyte Physiol* 259: F408–F414, 1990.
30. **Silva GB, Garvin JL.** Different patterns of stimulation of protein kinase C isoforms by intracellular and extracellular superoxide. *J Am Soc Nephrol* 17 (F-PO640), 473 A. 2006.
31. **Silva GB, Garvin JL.** Angiotensin II-dependent hypertension increases Na transport-related oxygen consumption by the thick ascending limb. *Hypertension* 52: 1091–1098, 2008.
32. **Silva GB, Garvin JL.** Rac1 mediates NaCl-induced superoxide generation in the thick ascending limb. *Am J Physiol Renal Physiol* 298: F421–F425, 2010.
33. **Silva GB, Ortiz PA, Hong NJ, Garvin JL.** Superoxide stimulates NaCl absorption in the thick ascending limb via activation of protein kinase C. *Hypertension* 48: 467–472, 2006.
34. **Tasken K, Aandahl EM.** Localized effects of cAMP mediated by distinct routes of protein kinase A. *Physiol Rev* 84: 137–167, 2004.
35. **Welch WJ.** Effects of isoprostane on tubuloglomerular feedback: roles of TP receptors, NOS, and salt intake. *Am J Physiol Renal Physiol* 288: F757–F762, 2005.
36. **Welch WJ, Wilcox CS.** Potentiation of tubuloglomerular feedback in the rat by thromboxane mimetic. Role of macula densa. *J Clin Invest* 89: 1857–1865, 1992.
37. **Zhou MS, Jaimes EA, Raij L.** Atorvastatin prevents end-organ injury in salt-sensitive hypertension: role of eNOS and oxidant stress. *Hypertension* 44: 186–190, 2004.