Regulation and function of renal medullary cyclooxygenase-2 during high salt loading

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1. ABSTRACT

Prostaglandins (PGs) are important autocrine/paracrine regulators that contribute to sodium balance and blood pressure control. Along the nephron, the highest amount of PGE_2 is found in the distal nephron, an important site for fine-tuning of urinary sodium and water excretion. Cylooxygenase-2 (COX-2) is abundantly expressed in the renal medulla and its expression along with urinary PGE_2 excretion is highly induced by chronic salt loading. Factors involved in high salt-induced COX-2 expression in the renal medulla include the hypertonicity, fluid shear stress (FSS), and hypoxia-inducible factor-1α (HIF-1 α). Site-specific inhibition of COX-2 in the renal medulla of Sprague-Dawley rats causes sodium retention and salt-sensitive hypertension. Together, these results support the concept that renal medullary COX-2 functions an important natriuretic mediator that is activated by salt loading and its products promote sodium excretion and contribute to maintenance of sodium balance and blood pressure.

2. INTRODUCTION

Increased salt intake is an important environmental factor contributing to the development of hypertension. Approximately 50% of hypertensive individuals are salt-sensitive (1). However, the molecular mechanism that determines salt sensitivity is poorly understood. The kidney is known to play a pivotal role in the maintenance of sodium balance and blood pressure. In particular, the renal medulla is capable to producing a number of natriuretic factors that are upregulated by salt loading and promote sodium excretion, thus eliciting natriuretic response (2-5). The impaired natriuretic response contributes to sodium retention and hypertension after high salt loading.

Prostanoids are products of arachidonic acid metabolism and their synthesis requires three sequential enzymatic reactions: release of arachidonic acid from membrane glycerophospholipids by phospholipase A_2, conversion of arachidonic acid to the unstable intermediate PGH_2 by cyclooxygenase-1 (COX-1) or cyclooxygenase-2 (COX-2), and isomerization of PGH_2 to a biologically active prostanoid by a specific prostaglandin synthase or thromboxane synthase (6). COX-1 and COX-2 share 66% homology in amino acid sequence and similar enzymatic properties but represent different cellular expression pattern and regulation (7-9). COX-1 is constitutively expressed in most tissues, whereas COX-2 shows low expression under baseline and it is induced in response to cytokines and other physiological or pathological stimuli (2). Prostaglandin E_2 (PGE_2) is the major prostaglandin produced in the kidney. Within the kidney, the highest amount of PGE_2 is found in the distal nephron (10). This nephron segment is also the primary target site for PGE_2 to regulate sodium transport (11-13). In general, renal medullary PGE_2 functions a natriuretic factor that inhibits sodium reabsorption and causes vasodilation, thus eliciting natriuresis (9). Renal medullary PGE_2 synthesis is also elevated after high salt loading (14). The enzymatic source of high salt-induced renal medullary PGE_2 synthesis appears to be COX-2. The expression of COX-2 in the renal medulla is induced by high salt loading (14,15) and its inhibition at systemic or tissue-specific manner produces salt-sensitive hypertension (14). The goal of this review to summarize the major findings in the literature concerning the regulation and function of renal medullary COX-2 during high salt loading. For general knowledge about renal PG pathway, you are referred to a number of previous review articles (9, 16-18).

3. DISCUSSION

3.1. Regulation of renal medullary COX-2 by high salt diet

PGE_2 is the major PG produced in the kidney, particularly in the renal medulla. Along the nephron, the highest amount of PGE_2 is detected in the inner medullary collecting duct (10). The action of PGE_2 also mainly takes place in the renal medulla. PGE_2 has been implicated in regulation of renal medullary blood flow, sodium and water transport in the CD, osmotic response, etc. It is well known that renal medullary PG synthesis is increased in response to salt loading. At basal condition, the expression of both COX-1 and COX-2 is predominantly expressed in the renal inner medulla. Our laboratory demonstrates that renal medullary COX-2 expression is markedly stimulated by a high salt diet whereas renal medullary COX-1 expression is unaffected (15) (Figure 1). These findings are consistent with the reports from other investigators (19-21). Interestingly, renal cortical COX-2 expression is stimulated by salt depletion (15, 22). These results represent strong evidence for distinct regulation of COX-2 in the different kidney regions (15, 23).

How salt loading stimulates renal medullary COX-2 expression is not known. Can high salt intake stimulate renal medullary COX-2 expression via increased local osmolality? Seven days of high salt (1% NaCl in drinking water) increased outer medullary osmolality from 362 +/- 13 to 423 +/- 6 mosmol/kg H_2O (24). We demonstrate that hypertonicity elevates COX-2 expression and PGE_2 release in murine IMCD cells. Osmotic regulation of COX-2 expression requires multiple members of the mitogen-activated protein kinase (MAPK) family, namely ERK1/2, p38, and c-Jun N-terminal kinase (JNK) (25). In these experiments, COX-2 induction by hypertonicity was partially inhibited by a blockade of each of these MAPKs and was completely blocked by simultaneous blockade of ERK1/2 and p38. In cultured renal medullary interstitial cells (RMICs), the COX-2 induction is dependent on NFκB (10). Interestingly, the activity of sirtutin 1 (Sirt1), a NAD-dependent deacetylase, upregulates COX-2 expression to protect RMICs from oxidant-induced cellular injury (26). Indeed, reactive oxygen species (ROS) are important cellular mediators during osmotic stress and are shown to mediate COX-2 upregulation in this setting (27). Accumulating evidence supports a renoprotective role of Sirt1 in various renal injury models including ischemia-reperfusion (28), diabetic nephropathy (29), and renal fibrosis (30). However, less is clear regarding a potential physiological role of Sirt1 in renal handling of salt and water or its interaction with COX-2. It is also interesting to note that Casali *et al.* reported that the decrease in the nuclear receptor peroxisome proliferator-activated receptor-γ (PPARγ) expression was required for hyperosmotic induction of COX-2 in renal epithelial cells MDCK. (31) Collecting duct PPARγ is shown to contribute to thiazolidinedione-induced fluid retention, a major side effect of the antidiabetic agent (32). It will be an interesting subject concerning the interaction between PPARγ and COX-2 in regulation of collecting duct function particularly during antidiuresis.

Increasing evidence suggests that fluid shear stress (FSS) stimulates COX-2 expression and PGE_2 release in the CD cells. Shear stress is well recognized to play an important role in regulation of vascular function and remodeling. FSS is a parallel friction force generated by flowing fluid and it occurs on the renal epithelial cells. It is surprising that the estimated shear stress for the tubular fluid in the CD is roughly the same as that with blood in small resistance vessels (0.2.-20 dyn/cm^2) (33). A series of studies from Garvin's laboratory demonstrates that luminal flow stimulates NO production in medullary thick ascending limb (mTAL) and that shear stress rather than pressure, cellular stretch, or ion delivery mediates flow-induced NO production (34) and that this is mediated by activation of transient receptor potential vanilloid 4 (TRPV4), a mechano-sensitive channel activated by luminal flow in different types of cells (35). The similar mechanism appears to operate in the CD.

FSS is shown to stimulate CD NO production (33) and endothelin-1 release (36) and both of these factors inhibit tubular Na^+ transport, thus promoting natriuresis. FSS-induced production of the natriuretic factors is considered to serve as a negative feedback mechanism to counterbalance flow-induced Na^+ reabsorption and K^+ secretion in the CD.

Recently, a series of studies from Rohatgi's laboratory examines the possibility that FSS elicited by increasing tubular flow induces the release of PGE_2 that modulates the ion transport in the CD. In cultured CD cells, FSS induced several fold increases in PGE_2 release that is dependent on intracellular Ca^{++} and ERK1/2 and p38, but not JNK (37). Interestingly inhibition of either COX-1 or COX-2 partially attenuates FSS-induced PGE_2 release, suggesting involvement of both COX isoforms. In this study, Na^+ and K^+ transport is determined in isolated perfused CCD exposed to increased flow in the presence or absence of indomethacin. At high flow rate, COX inhibition enhances flow-stimulated Na^+ reabsorption and abolishes flow-stimulated K^+ secretion. Interestingly, at low flow rate, COX inhibition was ineffective. These results suggest that PGE_2 regulation of CD ion transport only occurs under high but not low urine flow condition. This notion is compatible with the view that PGs may have little role in regulation of renal function under basal condition but can become important during physiological or pathological stimuli.

A subsequent study from Rohatgi's laboratory defines the role and regulatory mechanism of COX-2 in FSS-induced signaling in the CD (38). Murine inner

medullary CD3 (IMCD3) cells exposed to FSS for 4 h induced a marked increase in COX-2 protein expression, which is nearly completely abolished by inhibition of neutral-sphingomyelinase (N-SM) with GW4869. In mice injected with isotonic saline to expansion volume and urine flow, renal medullary COX-2 protein expression is elevated but renal cortical COX-2 expression remains constant; COX-1 expression is not affected in either cortex or medulla. Another study from Rohatgi's laboratory suggested repressed FSS-induced COX-2/PGE_2 synthesis as a potential mechanism for hypercholesterolemia associated essential hypertension (eHTN). (39) This study further confirmed the previous results that only high flow increased PGE_2 release in CCDs from normal diet fed mice, which was repressed in CCDs from 1% cholesterol diet fed mice CCDs. Moreover, cholesterol extraction in a CD cell line induced COX-2/PGE_2 release via p38 pathway activation.

Hypoxia-inducible factor-1α (HIF-1α) has been implicated as a potential transcriptional regulator of renal medullary COX-2 during chronic salt loading. It is well known that the pO_2 in the medulla is substantially lower than that in the cortex. Renal medullary pO_2 is detected in the range of 10 mmHg (40) although there is wide discrepancy in the results from different studies (41, 42). It is conceivable that renal medullary cells need to be equipped with oxygen-sensitive machinery in order to function in the precarious hypoxic environment. HIF-1α, a master regulator of genes involved in adaption to hypoxia, is abundantly expressed in the medulla. In response to chronic salt loading, renal medullary HIF-1α level is upregulated (43-45) as a result of reduced expression of HIF prolyl-hydroxylase-2 (PHD2), an enzyme that degrades HIF-1 Inhibition of HIF-1α in the rat renal medulla by local delivery of decoy oligodeoxynucleotides (ODNs) impairs natriuretic response, leading to sodium retention and elevation of arterial pressure from 118 + 1.9. to 154 + 6.3. mmHg after a high salt diet (44). This result provides the first functional evidence for antihypertensive action of renal medullary HIF-1α. Defective HIF-1α system is subsequently shown to be responsible for the pathogenesis of salt-sensitive hypertension in Dahl salt-sensitive hypertensive Rats (Dahl S) (46). Dahl S rats fail to exhibit high-salt-induced inhibition of PHD2 and subsequent activation of HIF-1α in the renal medulla. When PHD2 shRNA is introduced to the renal medulla of Dahl S rats, pressure natriuresis is restored and salt-sensitive hypertension is blunted (46). Conversely, overexpression of PHD2 in the renal medulla of uninephrectomized rats leads to suppressed renal medullary HIF-1α levels, blunted pressure natriuresis, and accelerated hypertensive response after a high salt diet (47). As a transcription factor, HIF-1α regulates transcription of a number of oxygen sensitive genes including nitric oxide synthase (NOS), COX-2, and heme oxygenase-1 (HO-1). The expression of these enzymes is upregulated after high salt intake and is responsible for production of various natriuretic mediators such as nitric oxide (NO), prostaglandins (PGs), and carbon monoxide (CO), which cause vasodilation and inhibition of tubular Na^+ transport, thus promoting sodium excretion and lowering blood pressure.

On the other hand, it was also reported that intracellular PGE_2 increased HIF-1α expression in human renal proximal tubular cells (HK2 cells). (48) Moreover, the study demonstrated that COX/PGE_2 mediated all-trans retinoic acid (ATRA) induced upregulation of HIF-1α in HK2 cells even in normoxia. Taken together, it seems that there is a crosstalk between HIF-1α and COX/PGE_2 pathway. They will enhance each other to play a protective role against hypertension or kidney diseases.

3.2. Function of renal medullary COX-2 during high salt loading

The impact of prostanoids upon blood pressure in humans is highlighted by the prohypertensive action of NSAIDs which nonselectively inhibit COX enzymes. NSAIDs are among the most widely prescribed drugs worldwide and are associated with sodium retention and hypertension, particularly in patients with preexisting hypertension (49-51). Although it is recently reported that celecoxib did not alter cardiovasucular and renal function during dietary salt loading with short-term, low-dose use of celecoxib in young to middle-aged adults, (52) most of clinical and animals' studies still support the theory that COX-2-derived prostanoids resist the development of fluid retention and hypertension (53). More than 50 clinical trials involving 13,000 subjects have shown that edema is among the most common side effects of COX-2 inhibition (54). Systemic administration of COX-2 inhibitors to experimental animals increases BP in a salt-dependent manner (55, 56). Along this line, COX-2 knockout mice spontaneously develop hypertension in a manner dependent on the background (57). Staehr's results also confirmed the increased BP in COX-2 knockout mice with no impairment in renal NO production in response to chronic high salt intake. (55, 56, 58) COX-2 inhibitors or the genetic absence of COX-2 markedly augments the constrictor actions of angiotensin II (59).

To address the potential roles of renal medullary COX-2 in the regulation of BP, we examined the effects of chronic intramedullary infusion of the COX-2 blocker NS-398 on blood pressure in uninephrectomized Sprague-Dawley rats during chronic salt loading (14). A 5-day intramedullary infusion of NS-398 resulted in <30 mmHg elevations of MAP whereas intravenous infusion of NS-398 produced a minimal effect on blood pressure (14). Zewde *et al* independently conducted the chronic intramedullary infusion experiments and observed the same phenomenon: site-specific inhibition of COX-2 in the renal medulla produces hypertension in animals fed a high-salt diet (60). The two studies are mutually
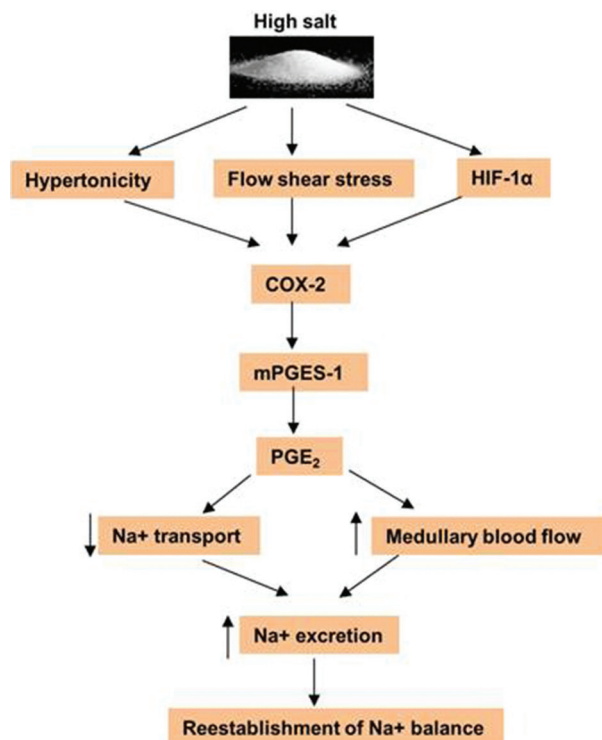
Figure 1. Illustration of the COX-2/mPGES-1/PGE2 pathway in high salt-induced natriuretic response. High salt loading induces renal medullary COX-2 expression via multiple mechanisms involving hypertonicity, flow shear stress, and HIF-1α. COX-2 couples with mPGES-1 to produce PGE2 to inhibit tubular Na$^+$ transport and increases medullary blood flow, resulting in increased urinary Na$^+$ excretion and reestablishment of Na$^+$ balance.

supportive and have strengthened the conclusion with regard to the antihypertensive function of renal medullary COX-2.

Despite the above-described antihypertensive role of renal medullary COX-2 during high salt loading, this pathway can be prohyertensive under other circumstances such as ANGII-induced hypertension. Our laboratory investigated the potential role of renal medullary COX-2 during angiotensin II (ANG II)-induced hypertension. *In vitro* and *in vivo* results suggested a crucial role of COX-2 in mediating upregulation of renal medullary (pro)renin receptor (PRR) expression and renin content during ANG II-induced hypertension. (18, 61). Our subsequent study further claimed that the specific COX-2/PGE$_2$/EP$_4$ pathway mediated the upregulation of renal medullary PRR expression by ANGII, leading to activation of intrarenal RAS and enhancement of the hypertensive response (62).

PGE$_2$ is a major prostanoid produced in the kidney (63). Among terminal PG synthases, prostaglandin E synthase (PGES) specifically catalyzes the conversion of PGH$_2$ to PGE$_2$. To date, at least three major forms of PGES have been identified. They are designated membrane-associated PGES (mPGES)-1, mPGES-2, and cytosolic PGES (cPGES) with mPGES-1 be the best characterized PGES (64). mPGES-1 couples with COX-2 to mediate PGE$_2$ production in response to inflammatory stimuli and mPGES-1 deletion blunts pain and inflammatory responses (65, 66). Besides inflammatory cells, mPGES-1 is widely expressed in adipose tissues, stomach, spleen, and kidney.

Emerging evidence suggests that this enzyme may play a role in regulation of sodium balance and BP (67-69). In this regard, following an acute enteral salt load, mPGES-1 KO mice exhibited impaired natriuresis as evaluated using metabolic cages (70). The KO mice were able to maintain a normal blood pressure on a standard high salt diet (71, 72) but were significantly hypertensive with increasing salt load (70), probably reflecting redundant mechanisms participating in renal salt handling. We found that within the kidney, mPGES-1 expression in wild-type mice predominates in the distal nephron where its expression is highly inducible by salt loading and that mPGES-1 KO mice exhibited blunted natriuretic response paralleled with remarkably suppressed NO and cGMP levels (70). We observed similar phenomenon in both ANG II-induced hypertension model (73) and DOCA-salt hypertension (74), in which mPGES-1 KO mice exhibited an enhanced hypertensive response compared to WT mice probably resulting from more severe oxidative stress. Zhang *et al.* further confirmed the vasodilatory and antihypertensive role of mPGES-1 during acute ANG II infusion. (75) Salazar FJ *et al.* employed a selective mPGES-1 inhibitor (PF-458) to study the renal effects of prolonged mPGES-1 inhibition. (76) The results showed mPGES-1 inhibition reduced renal blood flow (RBF) in dogs with low salt intake (LSI), but did not alter glomerular filtration rate (GFR) or renal hemodynamics. The renal effects of mPGES-1 inhibition were modulated by a compensatory increment in PGI$_2$. Together, these results suggest that mPGES-1 may couple with COX-2 to mediate PGE$_2$ production to modulate renal function.

4. SUMMARY AND PERSPECTIVE

In summary, COX-2 is abundantly expressed in the renal medulla where its expression is remarkably induced by chronic salt loading. Inhibition of COX-2 in the renal medulla causes sodium retention and salt-sensitive hypertension. Existing evidence from some, but not all studies favor mPGES-1 as a potential PGE synthase acting downstream of COX-2 in the renal medulla. Other sources of renal medullary PGE$_2$ production during high salt loading certainly warrant further investigation. Future studies also need to define specific EP receptors and their signaling pathways responsible for eliciting the natriuretic response.

5. ACKNOWLEDGEMENT

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