Microsomal prostaglandin E synthase-1 and blood pressure regulation

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Prostaglandin E $(PGE)_2$ is a major arachidonic acid metabolite in a wide variety of tissues and is implicated in the control of inflammatory as well as physiological responses. At least three major forms of PGE synthase (PGES) have recently been cloned and characterized: membrane-associated PGES (mPGES)-1, mPGES-2, and cytosolic PGES (cPGES). Among them, mPGES-1 is highly inducible by cytokine and is critically involved in pain and inflammatory responses. Emerging evidence suggests that mPGES-1 may also participate in blood pressure (BP) regulation through an impact on renal and vascular functions. Within the kidney, mPGES-1 predominates in the distal nephron where its expression is highly inducible by salt loading. Mice lacking mPGES-1 exhibit blunted natriuretic response paralleled with remarkably suppressed nitric oxide production, leading to salt-sensitive hypertension. These mice also exhibit an exaggerated hypertensive response to angiotensin II infusion. Together, these results suggest that mPGES-1 may be an important physiological regulator of BP.

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The E series of prostaglandins (PGEs) are the major products of arachidonic acid metabolism and are implicated in a wide variety of physiological and pathological processes. PGE_2 is a major mediator of inflammation and is also a key player in the control of various physiological functions.[1] The biosynthesis of PGE_2 requires three sequential steps of the cyclooxygenase (COX) pathway: the release of arachidonic acid from membrane glycerophospholipids by phospholipase A_2, conversion of arachidonic acid into the unstable intermediate PGH_2 by COX-1 or -2, and isomerization of PGH_2 to PGE_2 by PE synthase (PGES).[2,3] The activity of PGES has been detected in both cytosolic and microsomal fractions of various cells. To date, at least three major forms of PGES have been cloned and characterized.[3] They are designated as membrane-associated PGES (mPGES)-1, mPGES-2, and cytosolic PGES (cPGES). These isoforms of PGES represent products of three distinct genes with marked differences in enzymatic properties, modes of expression, and cellular and subcellular localizations, suggesting distinct functions.[3]

The first PGES (now referred to as mPGES-1) was cloned from human cells as microsomal glutathione S-transferase 1-like 1 whose PGE synthesis activity was dependent on glutathione.[4] This was followed by the isolation of the mouse, rat, and zebrafish homologs.[5–8] mPGES-1 is a member of the membrane-associated proteins in eicosanoids and glutathione metabolism superfamily, which also contains 5-lipoxygenase-activating protein and leukotrane C4 synthase.[6] The enzyme is a 16-kDa membrane-bound protein encoded by a 2.0-kb transcript. Like COX-2, mPGES-1 expression in inflammatory cells is highly inducible by proinflammatory stimuli. Several recent studies employing mPGES-1-deficient mice demonstrate a major role of mPGES-1 in pain and inflammatory responses.[9,10] By contrast, the physiological function of this enzyme has been studied in much less detail. Recent evidence from our group suggests that mPGES-1 may play an important role in facilitating renal salt excretion as well as in modulating the response to angiotensin (Ang) II.[11]

mPGES-2 is a second mPGES that was originally purified from bovine heart and subsequently cloned.[12,13] It is a 43-kDa protein containing a unique N-terminal hydrophobic domain, which determines the association with Golgi membrane. This enzyme is constitutively expressed in various cells and is considered to be functionally coupled with COX-1 and -2.

cPGES was originally identified from rat brain as a cytosolic glutathione-dependent PGES. It was identical to p23, which is a steroid hormone receptor/hsp90-associated protein.[14] The enzyme was constitutively expressed in the cytosol in a wide variety of cells and tissues and was unaltered by proinflammatory stimuli except that it was induced in rat brain following lipopolysaccharide treatment. The fact that cPGES and COX-1 are colocalized to the cytosol and also share the same constitutive properties favors a functional coupling between the two enzymes.

RENAL EXPRESSION OF mPGES-1

mPGES-1 is abundantly expressed in the kidney with a substantially higher level in the renal medulla than in renal cortex,[15] a pattern similar to COX-1 and -2.[16] A significant number of published studies using immunohistochemistry, *in situ* hybridization, and reverse transcriptase-polymerase chain reaction on microdissected nephron segments all consistently documented the predominant expression of mPGES-1 in the whole collecting duct (CD) system of mouse,[17] rat,[18] and rabbit.[19] Within the CD, the immunoreactive labeling was restricted to the principle but not intercalated cells.[19] This corresponds to the observations that the CD possesses the greatest rate of PGE_2 synthesis of any segment along the nephron and that there are well-established inhibitory effects of PGE_2 on Na^+ and water transport in the CD.[20] Double labeling studies using *in situ* hybridization and immunostaining demonstrated that mPGES-1 predominantly colocalizes with COX-1 in the CD, suggesting a functional coupling between the two enzymes in the distal nephron. In line with this notion, mPGES-1- and COX-1-deficient mice both develop hypertension when fed a high-salt diet.[11,21] A low level of mPGES-1 expression was inconsistently found in renal medullary interstitial cells[11,15,19] where COX-2 is abundantly expressed. This makes less likely that mPGES-1 couples with COX-2-mediating PGE_2 synthesis in renal medullary interstitial cells. This statement is not necessarily inconsistent with the notion that COX-2-derived prostanoids may be important regulators of BP during high salt intake.[21] It is possible that COX-2 may be linked with other PGES isoforms (e.g., cPGES or mPGES-2) than mPGES-1 to sustain PGE_2 synthesis in renal medullary interstitial cells in response to high salt loading.

Emerging evidence suggests that mPGES-1 expression in the CD is further upregulated in response to chronic salt loading.[11] In this study, the total abundance of mPGES-1 protein, as determined by immunoblotting, significantly increased in both cortex and the inner medulla following a 7-day high-salt diet. The increased expression was restricted to the CD as determined by immunostaining. In primary CD cells, hypertonic NaCl exerted a direct stimulatory effect on mPGES-1 expression. The ion-substitution experiments demonstrated that the stimulation was mediated by extracellular Cl^- and not Na^+. In general, these results suggest a physiological role of mPGES-1 in the CD.

The macula densa is a specialized cell type critically important for transducing the signal from the luminal electrolyte concentration to the afferent arterioles. There is mounting evidence that COX-2-derived PGE_2 participates in the signaling transduction in the macula densa. Therefore, attempts have been made to link this phenomenon with mPGES-1. Subsequently, mPGES-1 expression was detected in the macula densa in the rabbit kidney by both immunohistochemistry and *in situ* hybridization despite some variations in the expression levels in different reports.[15,19] Furthermore, mPGES-1 expression in the macula densa was responsive to the known stimuli for COX-2, including salt depletion and Ang-converting enzyme inhibition.[19] In general agreement with this finding, mPGES expression was induced in parallel with COX-2 in the macula densa in children with Bartter's syndrome.[22] Together, these studies suggest a possibility that mPGES-1 may functionally couple with COX-2 in the macula densa control of renin secretion. However, this notion is inconsistent with several other studies, which failed to detect mPGES-1 expression in the macula densa. In this regard, neither immunostaining nor *in situ* hybridization studies were able to detect the signal in the macula densa in the mouse kidney.[11,17] mPGES-1 mRNA was also undetectable in microdissected glomerulus with attachment of the macula densa from Sprague–Dawley rats although this preparation contained abundant signals of COX-2 and Na^+-K^+-$2Cl^-$ cotransporter.[18] It is unclear whether differences in species or sensitivities of the detection systems could account for the disparities among different reports. This calls for a need for further clarification of the expression and function of mPGES-1 in the macula densa cells.

In contrast to the detailed analysis of mPGES-1 expression in the kidney, the expression of the other two isoforms of PGES is relatively less studied. By *in situ* hybridization, mPGES-2 mRNA was detected in substantially higher levels in the renal cortex than in the renal medulla in the mouse kidney and by immunohistochemistry mPGES-2 protein was ubiquitously expressed along the nephron except that it was detected at much lower levels in the glomeruli.[23] The renal expression of cPGES was studied only by *in situ* hybridization and the signal was diffusely expressed in all nephron segments.[24] The diffused expression patterns of mPGES-2 and cPGES are apparently different from the localized distribution of COX-1 and -2 in the kidney. Therefore, the functional roles of mPGES-2 and cPGES in the kidney still remain elusive.

mPGES-1 AND BP

PGE_2 is an important autocrine/paracrine factor that contributes to sodium balance and BP control through mechanisms that primarily involve the regulation of renal excretory function and vasculature tone.[1] Recently, mPGES-1 has been identified in the renal distal nephron and the vasculature. These findings raise a possibility that mPGES-1 may participate in BP regulation through an influence on renal and vascular functions. To examine this possibility,

mPGES-1 −/− mice were placed on a 7-day high-salt diet plus saline as drinking fluid and mean arterial pressure was monitored by telemetry. Following chronic salt loading, the −/− mice developed more severe hypertension as compared to the +/+ controls (Figure 1a). This result is consistent with the observation that the −/− mice exhibited an impaired ability to excrete a sodium load in both acute and chronic settings.[11] It is highly possible that the abnormal salt handling accounts for the hypertensive phenotype. Given the nature of systemic deletion of the mPGES-1 gene, the site of action of mPGES-1 still cannot be determined. However, several lines of evidence point to the distal nephron. First, the induction of mPGES-1 in response to high salt loading was restricted in the entire CD system but not other nephron segments. Second, the CD is well known to be a major site of both production and action of PGE_2. These findings indicate an autocrine mode of action of mPGES-1 in the distal nephron. mPGES-1 may also act in a paracrine manner in which PGE_2 produced from the CD may modulate the transport function in the thick ascending limb and may also augment local blood flow by dilating the smooth muscle cells of vasa recta. Our results, however, are somewhat inconsistent with the report by Cheng *et al.*[25] who were unable to observe an elevated BP in mPGES-1 knockout mice on a high-salt diet. It is unclear whether differences in experimental protocols or genetic backgrounds may account for the disparities between the two studies.

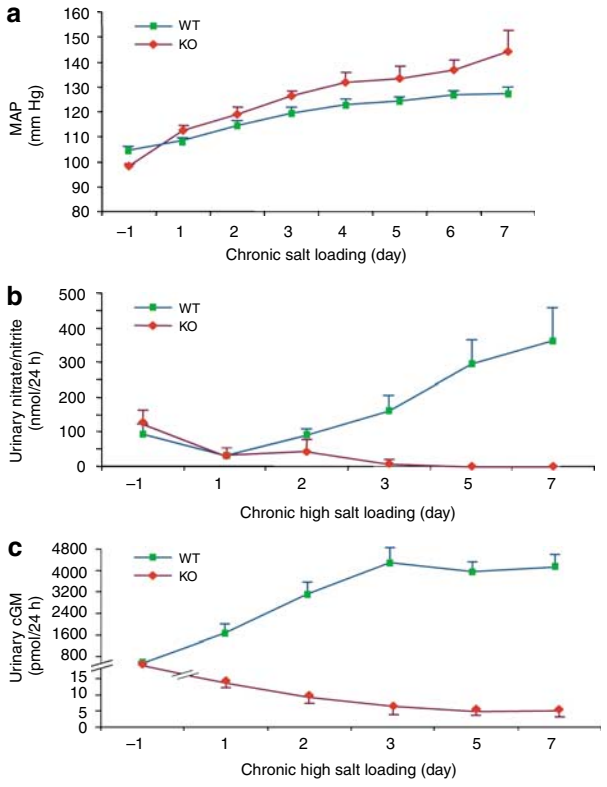

Figure 1 | Phenotype of mPGES-1 KO mice on a high salt diet. Daily (**a**) mean arterial BP (MAP), (**b**) urinary excretion of nitrate/nitrite, and (**c**) cyclic guanosine monophosphate (cGMP) in mPGES-1 +/+ (wild-type (WT)) and −/− (knockout (KO)) mice on a 7-day high-salt diet. MAP was determined by telemetry, urinary nitrate/nitrite by the Griess method, and urinary cGMP by enzyme-linked immunosorbent assay. (**a**) $P = 0.045$; (**b**) $P = 0.0003$; (**c**) $P < 0.0001$ (analysis of the interaction (time × strain) by repeated measures analysis of variance) (figure modified from Jia *et al.*[11]).

In line with the notion that PGE_2 functions as a natriuretic factor in the distal nephron, chronic salt loading is reported to increase renal synthesis of PGE_2. In the 1970s, an acute saline infusion was shown to increase prostaglandin-like materials in renal venous blood and medulla of canine kidney.[26] In the 1980s, a significant number of studies in rodents quite consistently showed that chronic salt loading elevated urinary excretion and renal medullary production of PGE_2.[21,27,28] The source and mechanisms of increased renal synthesis of PGE_2 during high salt intake have puzzled the field for more than 30 years. There is even a debate that increased urinary PGE_2 excretion during high salt loading might be related to increased urine flow. This issue has been partially resolved by the experiments employing mPGES-1 −/− mice which exhibit a remarkable blockade of high salt-induced urinary PGE_2 excretion.[11] The almost exclusive localization of high salt-induced mPGES-1 expression in the CD indicates that the increased renal PGE_2 synthesis may occur primarily in the distal nephron via mPGES-1.

The biological action of PGE_2 is mediated by G protein-coupled E-prostanoid receptors designated EP_1, EP_2, EP_3, and EP_4.[29] These four subtypes of EP receptor couple to distinct signaling pathways. In general, the EP_1 receptor signals via intracellular Ca^{2+} and phosphatidylinositol–bisphosphate hydrolysis, whereas the EP_2 and EP_4 receptors are coupled to Gs and signal by intracellular cyclic adenosine monophosphate. The EP_3 receptor is more complex in that it exists in multiple slice isoforms which display differential coupling to distinct signaling mechanisms via Gi (inhibition of cyclic adenosine monophosphate formation), Gs (stimulation of cyclic adenosine monophosphate formation), and Gq (stimulation of intracellular Ca^{2+} release). Specific EP receptors mediating the natriuretic action of mPGES-1-derived PGE_2 still remains elusive. The EP_1 receptor mRNA is detected predominantly in the CD where activation of this receptor inhibits sodium and water reabsorption via a Ca^{2+}-coupled mechanism. This evidence favors the EP_1 as a candidate EP receptor responsible for the well-documented natriuretic and diuretic effects of PGE_2. However, this notion is somewhat incompatible with a recent finding that mice lacking the EP_1 exhibit reduced but not enhanced urine-concentrating capability.[30] The EP_4 receptor is primarily expressed in the glomerulus and according to some, but not all, studies is found in the CD. Despite evidence for a potential role of EP_4 receptor in furosemide-induced natriuretic and diuretic responses,[31] a specific role of this receptor in the CD remains elusive. The EP_3 receptor is abundantly expressed in the renal medulla and the CD and mice lacking the EP_3 exhibit normal urinary concentrating function. Together, the net *in vivo* contribution of these three EP receptors to the control of sodium balance and BP is

unclear and awaits analysis of the respective EP knockout mice challenged with salt loading. The EP_2 predominates in the vasculature but not the CD, and mice lacking this receptor develop salt-sensitive hypertension, a phenotype similar to mPGES-1 knockout mice. These results suggest that the EP_2 may in part mediate the natriuretic and antihypertensive action of mPGES-1-derived PGE_2. The results also indicate a possible paracrine mode of action of PGE_2 in which mPGES-1-derived PGE_2 is released from the CD and acts on the EP_2 in the blood vessels.

Further evidence in support of the role of mPGES-1 in BP regulation came from the observation that mPGES-1 −/− mice develop an exaggerated hypertensive response to chronic Ang II infusion as compared with wild-type controls.[11] This finding may indicate the vascular action of mPGES-1. In line with this notion, this enzyme has been detected in the vasculature both *in vivo* and *in vitro* despite some discrepancies regarding its presence in vascular smooth muscle versus endothelial cells. As compared with the well-recognized vasodilatory action of prostacyclin, the vascular effects of PGE_2 have not been well appreciated. This is partially due to the fact that PGE_2 can be either vasodilatory or vasoconstrictory depending on the activation of distinct EP receptors and the net *in vivo* effect of PGE_2 might become unpredictable. The hypertensive phenotype of mPGES-1 −/− mice in the setting of Ang II infusion represents an *in vivo* evidence for the overall vasodilatory and antihypertensive function of endogenous PGE_2.

mPGES-1 AND NO

Within the kidney, the renal medulla has the greatest capacity for synthesis of PGs and nitric oxide (NO), the two important regulators of distal tubular fluid reabsorption. In line with this notion, the two isoforms of COX (COX-1 and -2) and the three isoforms of nitric oxide synthase (NOS) (neuronal NOS, inducible NOS, and endothelial NOS) are all predominantly expressed in the renal medulla where most of these enzymes except COX-1 can be induced by a high-salt diet. Functional studies indicate that renal medullary PGs[21,32] and NO[33,34] exert similar natriuretic and diuretic effects to stabilize BP during salt loading. The interaction has also been demonstrated in renal hemodynamic responses to bradykinin, norepinephrine, and Ang II.[35,36] If operation of a renal medullary depressor–natriuretic mechanism relies on the action of both PGs and NO, the question arises as to how the actions of these two autocrine/paracrine factors are coordinated. Existing literature indicates a mutual interaction between the two pathways. It is well documented that NO exerts a direct stimulatory effect on PGE_2 synthesis in a variety of cell types mostly through a COX-2-dependent mechanism. NO can activate COX-2 at levels of gene expression as well as enzyme activity. On the other hand, evidence demonstrates that PGE_2 stimulates NO formation, mostly in nonrenal cells. In this regard, PGE_2 augments neuronal NOS and endothelial NOS activities at post-translational levels in the spinal cord[37] and in cultured human vein endothelial cells,[38] respectively. The mutual interaction of PGE_2 and NO has been observed in renal cells as well, especially in the CD cells where NO stimulates COX-2 expression and PGE_2 release through mitogen-activated protein kinases[39,40] while PGE_2 enhances cytokine-induced NO formation probably through cyclic adenosine monophosphate.[41] These *in vitro* studies indicate that the two signaling pathways might be mutually stimulatory in the distal nephron as in other nonrenal cells.

A breakthrough has been made in understanding the *in vivo* relationship between PGE_2 and NO using mPGES-1 −/− mice.[11] As expected, chronic high salt loading gradually and significantly increased urinary nitrate/nitrite excretion in mPGES-1 +/+ mice with a maximal increase of 15-fold on day 7. In a sharp contrast, this increase was not seen in mPGES-1 −/− mice and urinary nitrate/nitrite excretion in these animals was even suppressed to undetectable levels after 3 days of high salt loading (Figure 1b). This result was further confirmed by measurement of urinary cyclic guanosine monophosphate (Figure 1c). Together, these findings reveal a linear relationship between PGE_2 and NO in the kidney at least in the setting of chronic salt loading. The precise location for PGE_2-dependent NO production under this condition is unknown but evidence points to the distal nephron. In this regard, the induction of renal mPGES-1 expression in response to high salt loading was restricted to the CD. Furthermore, high salt-induced expression of neuronal NOS, a major NO-producing enzyme in the CD was almost completely abolished in mPGES-1 −/− mice. These findings indicate that PGE_2 may regulate sodium and water transport in the CD via NO (Figure 2). The EP receptor

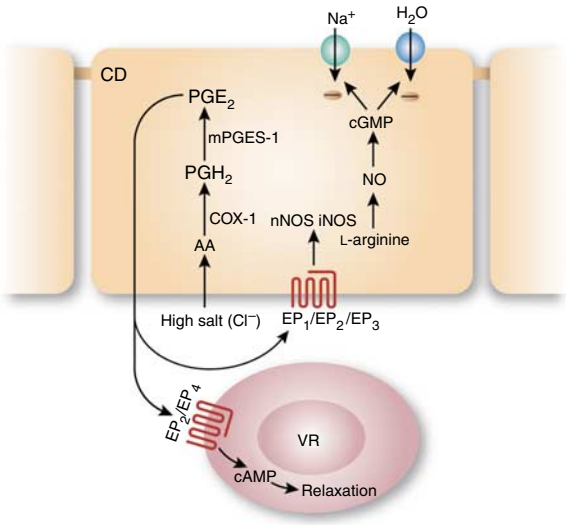

Figure 2 | Schematic illustration of mechanisms of renal tubular and vascular effects of mPGES-1-derived PGE_2. In response to salt loading, mPGES-1 couples with COX-1, mediating increased PGE_2 synthesis in the CD. The released PGE_2 can bind to G-protein-coupled EP receptors such as EP_1, EP_3, and EP_4 in the CD, activating neuronal NOS and inducible NOS that produce NO that inhibits Na^+ and water transport. PGE_2 can also act on EP_2/EP_4 receptors in the neighboring blood vessels to augment local blood flow. VR, vasa recta.

subtype responsible for stimulation of NO formation in the CD still remains elusive. Among the four EP receptor subtypes, the EP_1 is well known to mediate the tubular effects of PGE_2 via elevation of intracellular Ca^{2+}. Whether or not the EP_1-elicited calcium signaling in the CD is linked to NO is unclear.

In summary, mPGES-1 has an established role in pain and inflammatory responses and emerging evidence suggests that this enzyme is also involved in the maintenance of normal BP. mPGES-1 is predominantly expressed in the distal nephron and is further upregulated by a high-salt diet, representing a major source of high salt-induced PGE_2 synthesis. This enzyme helps stabilize BP by facilitating renal sodium excretion through a mechanism involving stimulation of NO synthesis. These results provide a new insight into the interaction of PGE_2 and NO in BP regulation.

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