mPGES-1 Protects Against DOCA-Salt Hypertension via Inhibition of Oxidative Stress or Stimulation of NO/cGMP

Zhanjun Jia, Toshinori Aoyagi, Tianxin Yang

Abstract—Microsomal prostaglandin E synthase-1 (mPGES-1) is a recently characterized cytokine-inducible enzyme critically involved in pain and inflammatory response. However, its role in blood pressure regulation is still debatable. The present study was undertaken to examine the effect of mPGES-1 deletion on DOCA-salt hypertension. After 2 weeks of DOCA plus 1% NaCl as drinking fluid, hypertension and sodium retention were more severe in mPGES-1 knockout (KO) mice than in wild-type (WT) controls. The indices of oxidative stress including urinary 8-isprostane and renal thiobarbituric acid-reactive substances were only modestly increased or unchanged in the WT mice but more significantly increased in the KO mice after DOCA-salt. Conversely, in response to DOCA-salt, the indices of antioxidant systems including renal expression of superoxide dismutase-3 and urinary nitrate/nitrite excretion were all significantly elevated in the WT mice but remarkably suppressed in the KO mice. Tempol treatment (50 mg/kg per day) in DOCA-salt KO mice produced a marked attenuation of hypertension, sodium retention, and kidney injury. Immunoblotting demonstrated increased renal expression of mPGES-1 in DOCA-salt WT mice. DOCA-salt induced a nearly 5-fold increase in urinary PGE2 excretion in the WT mice, and this increase was completely abolished in the KO mice. Together, these results suggest that mPGES-1-derived PGE2 confers protection against DOCA-salt hypertension likely via inhibition of oxidative stress or stimulation of superoxide dismutase-3 and urinary nitrate/nitrite system.

Key Words: cGMP ■ DOCA-salt hypertension ■ mPGES-1 ■ nitric oxide ■ oxidative stress

Prostaglandin E2 (PGE2) is a major product of arachidonic acid metabolism, being implicated in pain and inflammatory responses and in regulation of various physiological functions. In particular, PGE2 is a natriuretic and diuretic factor and therefore is antihypertensive. To date, at least 3 major forms of prostaglandin E synthase (PGES) have been cloned: membrane-associated PGES (mPGES)-1, mPGES-2, and cytosolic PGES. Mice deficient in mPGES-1 but not mPGES-2 or cytosolic PGES suppress PGE2 production, suggesting that mPGES-1 may represent the only enzymatic pathway capable of generating PGE2 in vivo. Several recent studies using mPGES-1 knockout (KO) mice demonstrate a major role of mPGES-1 in pain and inflammatory responses. The cardiovascular consequences associated with COX-2 inhibitors have stimulated the interest in mPGES-1 as a potential target of the next generation of anti-inflammatory drugs. Therefore, it is critically important to determine whether mPGES-1 plays a physiological role in the cardioenral system similar to COX-2.

The use of a synthetic mineralocorticoid, DOCA, which is an aldosterone analog, together with a high-salt diet (DOCA-salt), is a well-established means of inducing hypertension. This hypertension model is relevant to human primary aldosteronism. The relevance to hypertension in the general population is recently suggested by a genome-wide association study that identifies single-nucleotide polymorphisms of a few genes responsible for human hypertension, one of which is CYP17A1-encoding steroid 17-α-hydroxylase, an enzyme necessary for biosynthesis of mineralocorticosteroids. In response to prohypertensive insults, many physiological systems, particularly those with natriuretic and diuretic properties, are activated to help reestablish sodium balance and stabilize blood pressure. Here we describe mPGES-1 as an important antihypertensive factor in DOCA-salt hypertension.

Materials and Methods

Animals

The mPGES-1-null mice were originally generated by Trebino et al and provided by John McNeish (Pfizer, Groton, Conn.). All protocols using mice were conducted in accordance the principles and guidance of the University of Utah Institutional Animal Care and Committee.

DOCA-Salt Hypertension Model

The 3- to 4-month-old male mPGES-1 wild-type (WT) and KO mice were subject to DOCA-salt treatment. Under anesthesia with isoflurane, a slow-release (21-day) 50-mg DOCA pellet was implanted subcutaneously through a mid-scapular incision. Sham-operated animals served as controls. Separate mPGES-1 KO mice were subcutaneously implanted with the DOCA pellet together with an
Table. Sequences of Oligonucleotides for Quantitative Polymerase Chain Reaction

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<th>Gene</th>
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osmotic mini-pump driving infusion of vehicle or Tempol (50 mg/kg per day). After the surgery, animals received 1% NaCl in drinking water and a normal salt diet. Once per week, mice were placed in metabolic cages for collection of 24-hour urine.

Blood Pressure Measurement

Systolic blood pressure was measured by a tail-cuff method using a Visitech BP2000 Blood Pressure Analysis System (Apex, NC). All animals were habituated to the blood pressure measurement device for 7 days. They all underwent 2 cycles of 20 measurements reordered per day for a minimum of 3 days. Mean arterial pressure was determined by telemetry as previously described.29

Quantitative Reverse-Transcription Polymerase Chain Reaction

Total RNA isolation and reverse-transcription were performed as previously described.28 Oligonucleotides were designed using Primer3 software (available at http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). The sequences of the oligonucleotides are shown in the Table.

Enzyme Immunoassay

Urine samples were centrifuged for 5 minutes at 10,000 rpm. Concentrations of PGE2 and 8-isoprostane were determined by enzyme immunoassay according to manufacturer’s instructions (Cayman Chemicals). Urine albumin was determined using a murine microalbuminuria enzyme-linked immunosorbent assay kit (EXOCELL).

Measurement of Thiobarbituric Acid-Reactive Substances

The measurement of plasma thiobarbituric acid-reactive substances (TBARS) was based on the formation of malondialdehyde by using a commercially available TBARS Assay kit (10009055; Cayman Chemical) according to the manufacturer’s instructions.

Measurements of Urinary Nitrate/Nitrite and Superoxide Dismutase-3

These assays were performed as previously described.29

Results

Measurements of Blood Pressure

Systolic blood pressure was initially monitored by tail-cuff (Figure 1A). The values were not different between the genotypes at baseline. After DCOA-salt, a small but significant increase in systolic blood pressure was detected in WT mice at day 7 and remained elevated at day 14. Greater increases in systolic blood pressure were detected in mPGES-1 KO vs WT mice at days 7 and 14 of DOCA-salt treatment. These results were subsequently confirmed by telemetry (Figure 1B). Over 14 days of DOCA-salt treatment, a modest reduction of body weight was observed in both genotypes (22.7±0.8 in WT per day 0 vs 21.6±0.8 g in WT per day 14; n=6; P>0.05; 23.5±1.0 in KO per day 0 vs 22.8±1.1 g in KO per day 14; n=6; P>0.05).

Sodium and Water Balance Studies

Metabolic studies were performed to compare sodium and water balance in mPGES-1 WT and KO mice during DOCA-
salt hypertension. Once per week, mice were placed in metabolic cages so that a 24-hour urine sample could be collected. Daily urine collections were not performed because mice started to lose weight when kept in metabolic cages for 2 to 3 days. In WT mice, increased sodium intake and output and sodium balance were detected at day 7 and remained elevated at the similar level at day 14 of DOCA-salt treatment (Figure 2A–C). Greater increases in sodium intake and output and sodium balance were detected in KO vs WT mice at day 7; these differences between the genotypes became more prominent at day 14 (Figure 2A–C). Changes of water intake and output (Figure 2D, E) followed the same pattern as that of sodium intake and output. Interestingly, despite increased water intake and output in the KO mice, water balance was not different between the genotypes (Figure 2F). After 14 days of DOCA-salt, plasma sodium concentrations remained unchanged in WT mice but significantly increased in the KO mice (Figure 3A). DOCA-salt KO mice exhibited a greater increase in plasma osmolality (Figure 3B) and a greater decrease in urine osmolality (Figure 3C).


Production of Reactive Oxygen Species and Nitric Oxide/Superoxide Dismutase-3 Expression

DOCA-salt treatment in WT mice led to a 10% increase in renal TBARS content without affecting urinary 8-isoprostane excretion. In contrast, greater increases in both parameters were observed in DOCA-salt KO mice (Figure 4A, B). Urinary nitrate/nitrite (NOx) and superoxide dismutase-3 (cGMP) excretion were significantly elevated in DOCA-salt WT mice, but these increases were absent in DOCA-salt KO mice (Figure 4C, D). Urinary cGMP excretion in DOCA-salt KO mice was even much lower than the baseline level of either WT or KO mice (Figure 4D).

To understand renal sources of oxidative stress, we examined renal expression of gp91phox and p47phox, the 2 major NADPH oxidase subunits, along with NOX1 and SOD1–3 by quantitative reverse-transcription polymerase chain reaction. DOCA-salt treatment induced parallel increases in renal gp91phox (2.2-fold; \( P<0.01 \)) and p47phox (1.7-fold; \( P<0.01 \)) and SOD3 (1.7-fold; \( P<0.05 \)) mRNA in WT mice without an
effect on NOX-1, SOD1, or SOD2. As compared with WT controls, the increases in SOD3 but not p47phox or gp91phox were significantly blocked in the KO mice (fold changes for SOD3, p47phox, and gp91phox in DOCA-salt KO mice were 1.1-fold, 1.5-fold, and 1.7-fold, respectively).

**Effects of Tempol on Blood Pressure, Sodium, and Water Balance**

A separate experiment was conducted to determine the effects of Tempol on DOCA-salt hypertension and sodium retention. Tempol administration to DOCA-salt KO mice significantly decreased systolic blood pressure, plasma sodium concentrations, sodium intake and output, and almost normalized sodium balance (Figure 5A–E). Similar attenuations were seen for water intake and urine output, and water balance remained stabilized (Figure 5F–H).

**Kidney Injury**

Morphologically, a similar degree of renal cortical tubular hypertrophy was noticed in the 2 genotypes after DOCA-salt. Whereas renal medullary tubular dilation after DOCA-salt was more prominent in the KO vs WT mice, possibly reflecting the changes in urine flow, strikingly, Tempol administration in DOCA-salt KO mice corrected renal medullary tubular dilation to the level in DOCA-salt WT mice (Figure 6, left). Urinary albumin excretion in WT mice was

![Figure 3](image3.png)

Figure 3. Plasma sodium concentration (A), plasma osmolality (B), and urine osmolality (C) in mPGES-1 WT and KO mice over 14 days of DOCA-salt. N=6 per group.

![Figure 4](image4.png)

Figure 4. Renal TBARS content (A), urinary 8-isoprostane excretion (B), urinary excretion of NOx (C), and cGMP (D) in mPGES-1 WT and KO mice after 14 days of DOCA-salt. N=6 per group.
significantly elevated by DOCA-salt. A trend of further increase in urinary albumin levels was detected in the KO mice \( (P<0.05) \). This increase was significantly attenuated by Tempol treatment (Figure 6, right).

**Renal Expression of mPGES-1 and Urinary PGE\(_2\) Excretion**

Immunoblotting detected increases in mPGES-1 protein expression in both renal cortex and medulla in WT mice after DOCA-salt treatment (Figure 7A). This was associated with a 5-fold increase in urinary PGE\(_2\) excretion. As compared with WT controls, the KO mice had lower baseline levels of urinary PGE\(_2\) that was unresponsive to DOCA-salt treatment (Figure 7B).

### Discussion

mPGES-1 has received much attention as a promising target for antiinflammatory drugs.\(^{30}\) The lesson from COX-2 inhibi-
In mice, irrespective of uninephrectomy. As previously reported, we found that DOCA-salt treatment in mPGES-1-null mice exacerbated DOCA-salt hypertension and restored sodium balance. We provide further evidence that increased renal expression of mPGES-1 represents a dominant source of enhanced renal PGE2 synthesis during DOCA-salt.

DOCA-salt hypertension is influenced by species with malignant hypertension in rats vs moderate hypertension in mice, irrespective of uninephrectomy. As previously reported, we found that DOCA-salt treatment in mPGES-1 WT mice induced modest hypertension as determined by both tail-cuff and telemetry. Sodium accumulation in these mice was less significant than that in the KO mice. As compared with WT controls, mPGES-1-null mice exhibited severe disturbance of sodium and water metabolism, as reflected by marked increases in sodium and water intake and output and plasma sodium concentrations after DOCA-salt.

The reduction of urinary osmolality after DOCA-salt was more prominent in the KO vs WT mice, suggesting that mPGES-1 deletion may also affect urine concentration ability, a likely explanation of the hypernatremia. Both tail-cuff and telemetry demonstrated severe hypertension in DOCA-salt mPGES-1-null mice. The exaggerated hypertensive response in the null mice is likely the consequence of sodium retention.

Oxidative stress is well-known to play an important role in the pathogenesis of DOCA-salt hypertension. In general, the degree of oxidative stress correlated with the magnitude of blood pressure changes in the present study. Despite the increased renal expression of p47phox and gp91phox, the reluctant oxidant stress as reflected by renal TBARS or urinary 8-isoprostane was only modest that paralleled with a small increase in blood pressure in DOCA-salt WT mice. One explanation is that this mouse strain may be resistant to DOCA-salt–induced oxidative stress because of effective antioxidant systems. The activation of the antioxidant systems is evidenced by increased renal SOD3 expression and urinary NOx/cGMP excretion. SOD represents one of major antioxidant systems. In mammals, 3 forms of superoxide dismutase are present: SOD1 (cytosolic), SOD2 (mitochondrial), and SOD3 (extracellular). Interestingly, despite increased renal SOD3 expression, the expression of SOD1 and SOD2 remained unchanged. To our knowledge, this is the first study to examine SOD expression profile in the DOCA-salt kidney. The demonstration of increased NOx in DOCA-salt WT mice agrees with a previous study showing increased renal NO production and renal endothelial NO synthase expression in DOCA-salt rats. In contrast to the WT controls, DOCA-salt mPGES-1-null mice displayed a marked increase in urinary 8-isoprostane and renal TBARS, accompanied by suppressed NOx/cGMP excretion. The heightened oxidative stress correlated with greater increases in blood pressure. More importantly, administration of Tempol produced a marked attenuation of oxidative stress, sodium retention, and hypertension, all supporting involvement of oxidative stress. This notion is also supported by the remarkable inhibition of NO/cGMP system in DOCA-salt KO mice. Of particular note, urinary cGMP excretion in these mice was suppressed to a level far below the baseline, a finding almost analogous to that in the null mice after high-salt loading. Overall, our study supports the concept that the balance of oxidative stress and NO/cGMP represents a critical determinant of blood pressure; mPGES-1 deficiency tips the balance toward oxidative stress, thus leading to exaggerated hypertensive response (Figure 8). However, the cause–effect relationship between oxidative stress and NO/cGMP in this model is still unclear. It is also important to note that the reduction of urinary cGMP was much greater than that of urine NOx in DOCA-salt KO mice. This finding suggests that in addition to NO, other natriuretic pathways may also contribute to PGE2-stimulated cGMP production in DOCA-salt hypertension. Renal expression of atrial natriuretic peptide, brain natriuretic peptide, and C-type natriuretic peptide, which are important activators of cGMP production, significantly increased in the DOCA-salt rat kidneys.

Figure 7. Stimulation of renal mPGES-1 expression by DOCA-salt. A, Immunoblotting of mPGES-1 in the kidney regions from control and DOCA-salt WT mice. Shown are representatives of at least 3 independent experiments. B, Urinary PGE2 excretion in mPGES-1 WT and KO mice after DOCA-salt. N = 6 per group.
In support of the protective role of mPGES-1 in DOCA-salt hypertension, renal mPGES-1 expression was significantly elevated in this model. Urinary PGE2 excretion, an index of renal synthesis, was significantly increased in WT mice and this increase was almost completely abolished in mPGES-1 KO mice, supporting this isomerase as a major source of enhanced renal PGE2 synthesis during DOCA-salt. These results are in agreement with our previous observations that renal mPGES-1 expression is stimulated by salt and water loading. Together, renal mPGES-1 expression seems to be particularly responsive to various volume expansion states under which mPGES-1–derived PGE2 elicits natriuresis and diuresis, thereby helping reestablish sodium balance and stabilize blood pressure.

It is interesting to note that sodium intake is significantly greater in the KO than in WT mice after DOCA-salt treatment, suggesting a possible central action of mPGES-1. Aldosterone is well-known to be a positive regulator of sodium appetite. It is an intriguing possibility that mPGES-

**Perspectives**

mPGES-1 inhibitors have the potential to serve as the next generation of analgesic drugs for treatment of pain and inflammatory diseases. It is of critical significance to understand the role of mPGES-1 under physiological and pathological conditions. The current study demonstrated that mPGES-1 deletion exacerbated sodium retention and hypertension induced by DOCA-salt. Therefore, mPGES-1 inhibitors may be, to some extent, associated with cardiovascular consequences seen with COX-2 inhibitors.

**References**


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