mPGES-1-derived PGE2 mediates dehydration natriuresis

Zhanjun Jia,1,∗ Gang Liu,1,2,∗ Ying Sun,1 Yutaka Kakizoe,1 Guangju Guan,2 Aihua Zhang,3 Shu-Feng Zhou,4 and Tianxin Yang1,5

1Department of Internal Medicine, Veterans Affairs Medical Center and University of Utah, Salt Lake City, Utah; 2Department of Nephrology, The Second Hospital of Shandong University, Jinan, China; 3Department of Nephrology, Nanjing Children’s Hospital, Nanjing Medical University, Nanjing, China; 4Department of Pharmaceutical Sciences, College of Pharmacy, University of South Florida, Tampa, Florida; and 5Institute of Hypertension, Sun Yat-sen University School of Medicine, Guangzhou, China

Submitted 20 October 2011; accepted in final form 3 November 2012

Jia Z, Liu G, Sun Y, Kakizoe Y, Guan G, Zhang A, Zhou SF, Yang T. mPGES-1-derived PGE2 mediates dehydration natriuresis. Am J Physiol Renal Physiol 304: F214–F221, 2013. First published November 21, 2012; doi:10.1152/ajprenal.00588.2011.—PGE2 is a neurotrophic factor whose production is elevated after water deprivation (WD) but its role in dehydration natriuresis is not well-defined. The goal of the present study was to investigate the role of microsomal prostaglandin E synthase-1 (mPGES-1) in dehydration natriuresis. After 24-h WD, wild-type (WT) mice exhibited a significant increase in 24-h urinary Na+ excretion accompanied with normal plasma Na+ concentration and osmolality. In contrast, WD-induced elevation of urinary Na+ excretion was completely abolished in mPGES-1 knock-out (KO) mice in parallel with increased plasma Na+ concentration and a trend increase in plasma osmolality. WD induced a 1.8-fold increase in urinary PGE2 output and a 1.6-fold increase in PGE2 content in the renal medulla of WT mice, both of which were completely abolished by mPGES-1 deletion. Similar patterns of changes were observed for urinary nitrate/nitrite and cGMP. The natriuresis in dehydrated WT mice was associated with a significant downregulation of renal medullary epithelial Na channel-α mRNA and protein, contrasting to unaltered expressions in dehydrated KO mice. By quantitative RT-PCR, WD increased the endothelial nitric oxide synthase (eNOS), inducible NOS, and neuronal NOS expressions in the renal medulla of WT mice by 3.9-, 1.48-, and 2.6-fold, respectively, all of which were significantly blocked in mPGES-1 KO mice. The regulation of eNOS expression was further confirmed by immunoblotting. Taken together, our results suggest that mPGES-1-derived PGE2 contributes to dehydration natriuresis likely via NO/cGMP.

mPGES-1; PGE2; dehydration natriuresis; cGMP; nitric oxide

DEHYDRATION LEADS TO THE DEPLETION of plasma volume and the increase of body fluid osmolality. Such changes trigger the homeostatic response to conserve the fluids through the thirst mechanism, vasopressin release, and potentiated renal reabsorption. During the early stage of dehydration, an important physiological response is an acute increase in urinary Na+ excretion, a phenomenon termed dehydration natriuresis. This response will help prevent the further rise of plasma Na+ concentration and extracellular toxicity. Dehydration natriuresis has been demonstrated in humans as well as in other mammalian species, including dog, sheep, rat, and mouse (1, 7, 18, 28, 31, 33, 36, 37). A number of factors such as neurohormonal, neurosensory, and mechanoreceptive stimuli (18, 21) can potentiate dehydration natriuresis. However, the precise mechanism of dehydration natriuresis is still incompletely understood.

PGE2 is a major prostanoid in the kidney and possesses a natriuretic property owing to its ability to inhibit Na+ transport in the distal nephron (3, 6, 39, 43). The elevation of urinary PGE2 in response to dehydration accompanied with increased renal medullary cyclooxygenase (COX)-2 expression has also been well-demonstrated (29, 41, 47). However, the functional implication of dehydration-induced renal PGE2 synthesis is still incompletely understood.

To date, three PGE synthases (PGES) have been cloned, including microsomal PGES-1 (mPGES-1), microsomal PGES (mPGES-2), and cytosolic PGES (cPGES), of which mPGES-1 is the best characterized PGES (19, 22, 24, 25, 38). Within the kidney, mPGES-1 is most abundantly expressed in the collecting duct (12), an important nephron site for both production and action of PGE2. A series of studies from our group reveal an important role of mPGES-1-derived PGE2 in promoting urinary Na+ excretion under various conditions of extracellular volume expansion (21, 22, 24). The goal of the present study was to employ mPGES-1 knockout (KO) mice to investigate mPGES-1-derived PGE2 in dehydration natriuresis.

METHODS

Animals. mPGES-1 wild-type (WT) and KO mice were originally generated by Trebino et al. (44). This mouse colony was propagated at the University of Utah and maintained on a mixed DBA/1lacJxC57/Bl6x129/Sv background. In all studies, 3- to 4-mo-old male mice were used. All protocols employing mice were reviewed and approved by the University of Utah Institutional Animal Care and Use Committee.

Establishment of dehydration natriuresis mouse model. Male mPGES-1 WT and KO mice were subjected to the 24-h water deprivation (WD) by removing the water bottles from the metabolic cages (Hatteras Instruments). The control groups of animals had free access to water and standard food, while the WD groups were provided only with standard food. Twenty-four-hour urine was collected. After the urine collection, mice were killed and the blood and kidneys were harvested. The urine electrolytes excretion, plasma osmolality, and Na+ concentration were measured. Kidney tissues were assayed for PGE2 content or subjected to quantitative (q)RT-PCR analysis of gene expression.

Enzyme immunoassay. Urine samples were centrifuged for 5 min at 10,000 rpm and diluted 1:1 with enzyme immunoassay buffer. Concentrations of urinary and kidney tissue PGE2 were determined by enzyme immunoassay (Cayman Chemical, Ann Arbor, MI) according to the manufacturer’s instructions. The urine cGMP (Cayman Chemical) and nitrate/nitrite (NOx; Cayman Chemical) levels were also determined following the manufacturer’s instructions.
qRT-PCR. Total RNA was isolated from renal tissues using TRIzol. One microgram of total RNA was denatured at 65°C for 5 min, and cDNA synthesis was then performed at 42°C for 1 h using Superscript reverse transcriptase (BRL, Gaithersburg, MD). Oligonucleotides were designed using Primer3 software (available at http://frodo.wi.mit.edu/). The sequences of the oligonucleotide primers in the public sequence are as shown in the Table 1. Quantitative (q) PCR amplification was performed using the SYBR Green Master Mix (Applied Biosystems) and the Prism 7500 Real-Time PCR Detection System (Applied Biosystems). Cycling conditions were 95°C for 10 min, followed by 40 repeats of 95°C for 15 s and 60°C for 1 min.

Immunoblotting. Lysates of the kidney medullary tissue were stored at −80°C until assayed. Protein concentrations were determined using a Coomassie reagent. An equal amount of the whole tissue protein was denatured at 100°C for 10 min, separated by SDS-PAGE, and transferred to nitrocellulose membranes. The blots were blocked overnight with 5% nonfat dry milk in Tris-buffered saline (TBS), followed by incubation for 1 h with mouse polyclonal antibodies against endothelial nitric oxide synthase (eNOS; BD Transduction Laboratories, cat. no. 610297), epithelial Na channel-α (ENaC) (StressMarq, cat. no. SPC-403D), and mouse monoclonal anti-β-actin (Sigma, cat. no. A1978). The blots were washed with TBS followed by incubation with goat anti-mouse horseradish peroxidase-conjugated secondary antibody for eNOS and β-actin. Immune complexes were detected using enhanced chemiluminescence methods. The immunoreactive bands were quantified using the Gel and Graph Digitizing System (Silk Scientific).

Data analysis. Data are summarized as means ± SE. Statistical analysis was performed using one-way ANOVA or Student’s t-test as appropriate. P < 0.05 was considered statistically significant.

RESULTS

Effect of mPGES-1 deletion on dehydration-induced natriuretic response. Dehydrated WT mice had increased urine Na+ (231.2 ± 19.8 vs. 159.2 ± 15.6 μmol/24 h, P < 0.01; Fig. 1A) and unaffected urine K+ excretion (285.4 ± 31.7 vs. 284.1 ± 27.7 μmol/24 h, P > 0.05) and urine Cl− excretion (290.98 ± 37.0 vs. 274.7 ± 30.3 μmol/24 h, P > 0.05) excretion (Fig. 1, B and C). However, in response to WD, the increase in urine Na+ output in the KO mice was completely blocked (128.6 ± 25.9 vs. 152.6 ± 17.3 μmol/24 h, P > 0.05; Fig. 1A), indicating the blunted dehydration natriuresis. WD reduced urine volume (0.72 ± 0.16 vs. 0.97 ± 0.1 ml, P < 0.05, n = 14–15) and elevated urine osmolality (2.658.1 ± 304.7 vs. 1.916.0 ± 157.1 mosmol/kgH2O, P < 0.01, n = 14–15) in WT mice. At baseline, neither urine volume nor urine osmolality was different between WT and KO strains. In contrast, in response to WD, the KO mice exhibited a smaller urine volume (0.4 ± 0.1 ml, P < 0.01 vs. WT/WD, n = 13) and higher urine osmolality (3,603.1 ± 180.7 mosmol/kgH2O, P < 0.01 vs. WT/WD, n = 13), suggesting enhanced urine concentrating ability.

Effect of mPGES-1 deletion on plasma sodium concentration and plasma osmolality after WD. Impaired dehydration natriuresis may lead to hypernatremia and increased plasma osmolality. We therefore measured plasma Na+ concentration and osmolality in both WT and KO mice after 24-h WD. Indeed, dehydrated mPGES-1 KO mice displayed a significantly higher plasma Na+ concentration (KO/WD 142.3 ± 1.42 vs. WT/WD 137.1 ± 1.9 mmol/l, P < 0.05; Fig. 2A) and a trend increase of plasma osmolality (KO/WD 284.0 ± 2.34 vs. WT/WD 280.0 ± 1.5 mosmol/kgH2O, P = 0.053; Fig. 2B). It is likely that elevated plasma Na+ concentration and osmolality in dehydrated mPGES-1 KO mice are the direct consequence of impaired natriuretic response.

Effects of mPGES-1 deletion on dehydration-induced renal PGE2 production. To evaluate mPGES-1 as a potential source of dehydration-induced renal PGE2 synthesis, we examined
urinary PGE₂ excretion and tissue PGE₂ content in mPGES-1 WT and KO mice after 24-h WD. WD in WT mice significantly increased urinary PGE₂ excretion (685.95 ± 158.8 vs. 376.0 ± 66.3 pg/24 h, P < 0.05; Fig. 3A) and this increase was absent in mPGES-1 KO mice (236.9 ± 58.1 vs. 221.8 ± 39.8 pg/24 h, P > 0.05; Fig. 3A). Interestingly, dehydration-induced increases in tissue PGE₂ content in WT mice were observed in the renal medulla (8.87 ± 1.2 vs. 5.45 ± 0.7 pg/μg protein, P < 0.05; Fig. 3B) but not in the renal cortex (1.1 ± 0.08 vs. 1.06 ± 0.14, P > 0.05; Fig. 3C). In contrast, the medullary induction of PGE₂ production was completely blocked in the KO mice (Fig. 3B). All these findings demonstrated that mPGES-1 is the predominant enzyme source of dehydration-induced renal PGE₂ synthesis.

**Effect of mPGES-1 deletion on renal ENaC expression after WD.** To test the possibility that PGE₂ may promote Na⁺ excretion by inhibiting ENaC expression in the distal nephron, we examined the regulation of mRNA expression of the three ENaC subunits after WD. By qRT-PCR, 24-h WD induced a 34% reduction of ENaC-α mRNA in WT mice, which was completely blocked in the KO mice (Fig. 5A). By Western blotting, WD resulted in a reduction of ENaC-α protein by 40% in WT mice (P < 0.05; Fig. 6, A and B), but without an effect in KO mice (Fig. 6, C and D). In contrast, renal mRNA expression of ENaC-β or γ was unaffected by WD, irrespective of the genotype (Fig. 5, B and C).

**Regulation of renal medullary expression of eNOS, iNOS, and nNOS by WD.** The altered urinary NOx and cGMP excretion in dehydrated mPGES-1 KO mice suggested a potential role of NO/cGMP system in mediating PGE₂-elicited dehydra-

---

**Fig. 2.** Effect of mPGES-1 deletion on plasma Na⁺ concentration and plasma osmolality after dehydration. A: plasma Na⁺ concentration. B: plasma osmolality. Control: n = 6–7. Dehydration: n = 7–9. Data are means ± SE.

---

**Fig. 3.** Effect of mPGES-1 deletion on dehydration-induced renal PGE₂ synthesis. A: 24-h urinary PGE₂ excretion. Control group: n = 7–8. Dehydration group: n = 11–13. B: PGE₂ content in the renal medulla; n = 6–9 per group. C: PGE₂ content in the renal cortex; n = 6–9 per group. Data are means ± SE.
Discussion

Dehydration natriuresis is an established physiological phenomenon in many mammalian species (1, 7, 18, 28, 31, 33, 36, 37) but the underlying mechanisms, especially the renal mechanisms, are poorly characterized. The present study was undertaken to define the role of PGE2, a well-known natriuretic factor, in the occurrence of dehydration natriuresis, by examining the phenotype of mPGES-1 null mice after WD. We found that 24-h WD in WT mice increased urinary Na excretion, accompanied with increased urinary PGE2 output and renal medullary PGE2 concentration. In contrast, mPGES-1 null mice displayed a nearly complete blockade of the increase in urinary Na⁺ excretion and renal PGE2 synthesis. As a result, the null mice developed hypernatremia and a trend increase in plasma osmolality. We further provide evidence that mPGES-1-derived PGE2 may elicit dehydration natriuresis through NO/cGMP. Overall, these results represent compelling evidence supporting an important role of mPGES-1-derived PGE2 in the occurrence of dehydration natriuresis.

Along the nephron, the distal nephron is the major site for both production and action of PGE2. PGE2 synthesis in the collecting duct cells is modulated by osmolality (20). Along this line, it is consistently demonstrated that renal medullary COX-2 expression is induced by WD (13, 45); the opposite is true in that the COX-2 expression is reduced by water loading or chronic furosemide infusion that eliminates renal medullary osmotic gradient (4). Extensive in vitro studies have defined the signaling transduction pathway leading to osmotic regulation of COX-2 in collecting duct and renal medullary interstitial cells that primarily involve the activation of MAPK and NF-κB (13, 46). However, the functional implication of dehydration-induced renal medullary PGE2 synthesis is still poorly understood. Renal medullary PGE2 produced under WD is thought to antagonize the antidiuretic action of vasopressin, preserve the medullary blood flow, and improve cell survival but the precise role of PGE2 is unclear. mPGES-1 is a novel isomerase possessing PGE2 synthesizing activity in vivo and in vitro. The availability of mPGES-1 null mice offers a unique opportunity to examine the in vivo function of PGE2. The present study for the first time demonstrates that mPGES-1-derived PGE2 serves as a key regulator of urinary Na⁺ excretion.
tion during WD. In a separate study (23), we demonstrate that mPGES-1 deletion enhances urine concentrating capability after WD via stimulation of renal aquaporin-2 expression. The dual role of PGE$_2$ facilitates the separate but coordinated regulation of salt and water handling at least during WD.

The NO/cGMP pathway represents the common mechanism triggering the natriuretic response under various physiopathological conditions. Indeed, dehydration natriuresis in WT mice is accompanied by elevated urinary NOX and cGMP excretion. Consistent with this finding, the mRNA levels of three NOS subtypes were upregulated in the medullary tissues of WT mice following WD, which are in a complete agreement with previous reports (30, 42). Strikingly, the elevation of urinary NO/cGMP along with the upregulation of NOS isoforms were completely abolished by mPGES-1 deletion. It is conceivable that PGE$_2$ may signal through the NOS/NO/cGMP pathway to elicit the natriuretic response during WD. This finding is also in accordance with a series of our previous reports of defective regulation of salt and water handling at least during WD.

Considering ENaC as the major Na$^+$ transport route in the distal nephron, it seems reasonable to speculate that ENaC may serve as a molecular target of PGE$_2$ during WD. Indeed, we found that WD in WT mice induced significant downregulation of ENaC-$\alpha$ without affecting ENaC-$\beta$ and ENaC-$\gamma$, which at least in part accounted for dehydration natriuresis. This down-regulation was completely prevented in mPGES-1 null mice, suggesting that PGE$_2$ may mediate dehydration natriuresis by targeting ENaC-$\alpha$. Deletion of ENaC-$\alpha$ without disruption of ENaC-$\beta$ and ENaC-$\gamma$ in the collecting duct and connecting tubule caused the significant sodium imbalance (8), indicating that alteration of single ENaC subunit can affect the activity of the whole channel activity. Interestingly, mPGES-1 KO mice exhibited increased baseline level of ENaC-$\alpha$ mRNA in the renal medulla, suggesting that mPGES-1-derived PGE$_2$ may tonically suppress ENaC expression. Of note, the constitutive elevation of ENaC-$\alpha$ expression in mPGES-1 KO mice apparently did not cause obvious disturbance of Na$^+$ balance at the basal condition. The reason for this phenomenon is unclear. The transcript change may not correlate with the channel activity or there might be some compensatory changes in other factors.
sodium channels or renal hemodynamic that may offset the influence of altered ENaC expression.

In summary, the present study examined the role of mPGES-1 in dehydration natriuresis by using mPGES-1 null mice. mPGES-1 deletion prevented dehydration natriuresis, leading to hypernatremia and a trend increase in plasma osmolality. This was associated with blockade of dehydration-induced increases in urinary and renal medullary PGE2 levels and in urinary NOx and cGMP excretion. Together, these results demonstrate that mPGES-1 mediates dehydration natriuresis likely through NO/cGMP pathway.

**Perspectives**

In light of the cardiovascular consequences associated with COX-2 inhibitors, there is rising interest in developing mPGES-1 inhibitors as the next generation of analgesics. It is of critical importance to understand the role of mPGES-1 in physiological processes. The present study supports the concept that mPGES-1 inhibition may lead to perturbation of sodium and water balance, particularly during WD.

**ACKNOWLEDGMENTS**

The authors thank Alexandra Panasiuk (University of Utah) and Maicy Downton (University of Utah) for administrative and technical assistance.

**GRANTS**

This work was supported by VA Merit Review, National Basic Research Program of China 973 Program 2012CB517600 (No.2012CB517602), and National Institutes of Health Grant DK079162. T. Yang is an Established Investigator from American Heart Association and Research Career Scientist in Department of Veterans Affairs.

**DISCLOSURES**

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

**AUTHOR CONTRIBUTIONS**


**REFERENCES**


Fig. 8. Regulation of renal medullary eNOS protein expression by dehydration. A: immunoblots of eNOS in the medulla of WT and KO mice. B: densitometric analysis of eNOS protein expression in WT mice. C: densitometric analysis of eNOS protein expression in KO mice. Control: n = 3–5. Data are means ± SE.


