Role of Microsomal Prostaglandin E Synthase 1 in the Kidney

Helene Francois,* Carie Facemire,* Anil Kumar,* Laurent Audoly,† Beverly Koller,‡ and Thomas Coffman*

*Divisions of Nephrology, Duke University and Durham Veterans Affairs Medical Centers, Durham, North Carolina, †MedImmune, Gaithersburg, Maryland, and ‡Department of Genetics, University of North Carolina, Chapel Hill, North Carolina

Prostaglandin E2 (PGE2) is one of the most ubiquitous prostanoids in the kidney, where it may influence a wide range of physiologic functions. PGE2 is generated through enzymatic metabolism of prostanoid endoperoxides by specific PGE synthases (PGES). Several putative PGES have been identified and cloned, including the membrane-associated, inducible microsomal PGES1 (mPGES1), which is expressed in the kidney. To evaluate the physiologic role of mPGES1 in the kidney, mice with targeted disruption of mPges1 gene were studied, with a focus on responses where PGE2 has been implicated, including urinary concentration, regulation of blood pressure, and response to a loop diuretic. The absence of mPGES1 was associated with a 50% decrease in basal excretion of PGE2 in urine (P < 0.001). In female but not male mPGES1-deficient mice, there was a reciprocal increase in basal excretion of other prostanoids. Nonetheless, urinary osmolalities were similar in mPges1+/− and mPges1−/− mice at baseline and after 12 h of water deprivation. Likewise, there were no differences in blood pressure between mPGES1-deficient and wild-type mice on control or high- or low-salt diets. The furosemide-induced increase in urinary PGE2 excretion that was seen in wild-type mice was attenuated in mPGES1-deficient mice. However, furosemide-associated diuresis was reduced only in male, not female, mPGES1-deficient mice. Stimulation of renin by furosemide was not affected by mPGES1 deficiency. These data suggest that mPGES1 contributes to basal synthesis of PGE2, but there are other pathways that lead to renal PGE2 synthesis. Moreover, there are significant gender differences in physiologic contributions of mPGES1 to control kidney function.

ated regional expression of mPGES1 in the kidney by in situ hybridization and immunocytochemistry (5,17–19). There is a general agreement among these studies that mPGES1 is expressed in the distal nephron from the connecting tubule through the cortical and medullary collecting duct (5,17–19). In this region, the distribution of mPGES1 parallels COX-1 expression (18,19). This distribution is consistent with previous studies indicating that the collecting duct is one of the nephron segments with the highest capacity for PGE2 generation (1); abundant EP receptors, including EP1, EP3, and EP4, are also expressed in the collecting duct (4,20). Moreover, this segment of the nephron plays a critical role in the final adjustments of sodium excretion that have a major impact on fluid volume and BP homeostasis. However, the capacity of individual mPGES to influence renal functions is not known.

In studies that used mPGES1-deficient mice, we show here that mPGES1 contributes to basal and stimulated PGE2 generation by the kidney. In male mice, augmented generation of PGE2 by mPGES1 contributes to furosemide-induced diuresis. However, the relative contribution of mPGES1 to synthesis and functional consequences of PGE2 in the kidney differs significantly between genders.

Materials and Methods

Animals

Production of mice with targeted disruption of the mPges1 gene has been described previously (21). The mPges1 mutation was originally generated by homologous recombination in embryonic stem (ES) cells that were derived from DBA/1 inbred mice. Resulting chimeras were crossed with DBA/1 mice (Jackson Laboratory, Bar Harbor, ME) to generate inbred DBA/1 mice that bear the mPges1 mutation. Inbred DBA/1 mPges1+/− and mPges1−/− mice that were generated by intercrossing DBA/1 mPges1+/− mice were used for the studies described here. Mice were bred and maintained in the animal facility of the Durham Veterans Affairs Medical Center, and genotypes were determined as described previously (21). The experimental procedures described next were approved by the respective institutional animal care and use committees of the Durham Veterans Affairs and Duke University Medical Centers.

Measurement of Systolic BP in Mice

Systolic BP was measured in conscious mice using a computerized tail-cuff system (Hatteras Instruments, Cary, NC) after 2 wk of daily training as described previously (22). Data were recorded at baseline for 2 wk and then 5 d a week throughout the study period (one set of 10 measurements per day). Measurements with a SD >20 mmHg for the systolic BP were discarded. This method was validated previously and correlates well with direct measurements of intra-arterial pressure (23). Baseline BP was measured on a control diet (0.4% NaCl). For determination of whether mPGES1 plays a role in BP homeostasis when dietary sodium intake is altered, BP was monitored while mice were fed low-salt (LS; <0.02% NaCl) or high-salt (HS; 6% NaCl) diets for 3 wk (Harlan Teklad, Madison, WI). For these studies, the experimental groups consisted of both male mPges1+/− (n = 14) and mPges1−/− (n = 11) and female mPges1+/− (n = 6) and mPges1−/− (n = 7) mice.

Measurements of Urinary Prostanoid Excretion

Urinary prostanooid excretion was measured using individual ELISA assays. For measurement of PGE2, fresh urine samples were incubated in phosphate and carbonate buffer prepared with deionized water and incubated overnight at 37°C to convert intact PGE2 and its intermediate metabolites to the stable PGE2 metabolite (PGEM). Concentrations of PGEM in urine were determined by using a specific ELISA assay (Cayman Chemicals, Ann Arbor, MI). Previous studies have shown that urinary PGEM is a reliable indicator of urinary PGE2 excretion in vivo (24). Thromboxane B2 (TxB2), the stable metabolite of TXA2, and 6-keto-PGF1α, the stable metabolite of prostacyclin (PGI2), were measured in fresh urine using specific ELISA (Cayman Chemicals).

Assessment of Urinary Concentrating Capacity

Mice were placed in metabolic cages for collection of urine and monitoring of water intake. After an initial adaptation period, water intake and urine volumes were measured while the mice had free access to water. Urine of male and female mPges1+/− and mPges1−/− mice was collected by bladder massage before and after a 12-h period of water deprivation. Urine osmolalities were then immediately measured using a vapor pressure osmometer (Wescor Instruments, Logan, UT) as described previously (25).

Responses to the Loop Diuretic Furosemide

A 5-mg/ml furosemide (Sigma Chemicals, St. Louis, MO) solution was prepared in 10% DMSO in deionized water at pH 7. The 10% DMSO solution alone was used for control injections. Mice were administered an injection of 0.1 ml/10 g body wt of the furosemide solution or vehicle control and were immediately placed into individual metabolic cages, where they were provided free access to tap water. Urine was collected for exactly 4 h. Urine volumes were measured using tared containers, and urine sodium concentrations were measured using a flame photometer (Instrumentation Laboratory, Lexington, MA). For definition of the contribution of prostanoids to the furosemide response, separate groups of inbred male and female mice were given diclofenac (10 mg/kg; Sigma Chemicals) in drinking water for 24 h before the furosemide injection.

Reverse Transcription and Real-Time Quantitative PCR

Total RNA was extracted from whole kidneys using TriReagent (Sigma) according to the manufacturer’s protocol. RNA was DNase-treated using Turbo DNA-free (Ambion, Austin, TX) to remove genomic DNA contamination. RNA yield was quantified by ultraviolet spectrophotometry, and integrity was verified by 1% agarose gel electrophoresis and staining with ethidium bromide. Only RNA with A260/280 >1.7 and displaying no significant degradation was used for reverse transcription.

cDNA were synthesized from 5 μg of total RNA using random hexamers and SuperScript II reverse transcriptase (RT; Invitrogen, Carlsbad, CA). “No RT” samples that lacked RT were prepared during each RT reaction for use as negative controls during PCR. Real-time quantitative PCR was performed using the fluorogenic 5′-exonuclease assay (26). Primers and dual-labeled probes (5′-FAM, 3′-TAMRA) targeting mPGES1 (assay ID Mm00452105_m1; exons 2 to 3 boundary, amplicon length 80 bp) were designed previously. cDNA and negative control (no RT, water) templates (1 μl) were added to 20 μl PCR reaction mixtures that consisted of 1× ABI TaqMan Universal PCR master mix and either 1×
human eukaryotic 18S rRNA primer-probe mix or 1× primer-probe mix for the gene of interest. Gene expression was quantified using the two standard curve method for relative quantification (27). Briefly, a five-point dilution series of positive control cDNA was prepared, and standard curves of threshold cycle versus relative template concentration were generated for the gene of interest and housekeeping gene (18S rRNA). Template concentrations of unknown samples were then determined, based on their threshold cycle values, from these standard curves. Expression of mPGE1, mPGE2, cPGE, COX-1, and COX-2 was normalized to 18S rRNA expression in the same tissue.

In a separate group of mice, furosemide or vehicle was administered as described. Four hours later, kidneys were harvested, RNA was extracted, and expression of mPGE1 and COX-2 was determined.

**Furosemide-Stimulated Renin Expression**

To examine the role of mPGE1 in regulation of renin, we compared baseline and furosemide-stimulated renin expression in male mPges1+/+ and mPges1−/− mice. Groups of mice were of given furosemide (2.5 mg/kg per d) in the drinking water (n = 12) or tap water alone (n = 7). After 5 d, kidneys were harvested, RNA was isolated, and renin mRNA levels were determined by real-time PCR as described.

**Statistical Analyses**

The values for each parameter within a group are expressed as the means ± the SEM. For comparisons between groups, statistical significance was assessed using ANOVA followed by unpaired t test adjusted for multiple comparisons. A paired t test was used for comparisons within groups.

**Results**

**mPGE1 and Urinary Prostanoid Excretion**

To determine the contribution of mPGE1 to urinary prostanoid excretion, we measured excretion of PGE2, TXA2, and PGI2 metabolites in urine that was collected from mPges1−/− and mPges1+/+ mice. In general, as shown in Figure 1, urinary excretion of all prostanoid moieties was significantly higher in female compared with male mice irrespective of mPges1 genotype (P < 0.001 for urinary PGEM excretion and TxB2 excretion between male and female mice; P < 0.05 for urinary 6 keto-PGF1α). Excretion of PGE2 metabolites was reduced by approximately 50% in male mPges1−/− mice compared with wild-type (WT) controls (from 368 ± 50 to 196 ± 34 pg/24 h; P < 0.01; Figure 1A); 24-h urine volume was similar in both groups (1.1 ± 0.6 versus 1.1 ± 0.5 ml). In contrast, urinary excretion of TxB2 (1579 ± 244 versus 1509 ± 253 pg/24 h) or 6 keto-PGF1α (1941 ± 417 versus 1422 ± 132 pg/24 h) were unaffected by the mPges1 mutation in male mice.

As shown in Figure 1B, PGE2 excretion was also reduced by approximately 50% in female mPges1−/− mice compared with controls (2527 ± 248 versus 1306 ± 217 pg/24 h; P < 0.01). However, basal excretion of PGE2 metabolites was almost fourfold higher in female mPges1−/− mice than in WT male mice (1306 ± 217 versus 368 ± 50 pg/ml; P < 0.01). Moreover, in contrast to the male mPges1−/− mice, urinary excretion of other prostanoid species was increased in the mPGE1-deficient female mice. For example, urinary TxB2 excretion was 5473 ± 635 pg/24 h in the mPges1−/− female mice compared with 3960 ± 377 pg/24 h in controls (P < 0.05). Similarly, excretion of 6 keto-PGF1α was 4088 ± 660 pg/24 h in the mPges1−/− female mice compared with only 2177 ± 250 pg/24 h in female controls (P < 0.05).

![Figure 1](image_url)

*Figure 1.* Microsomal prostaglandin E synthase 1 (mPGE1) and urinary prostanoid excretion. Prostanoid excretion was determined in 24-h urine collections from male wild-type (WT; n = 15) and mPges1−/− (n = 11) mice (A) and female WT (n = 6) and mPges1−/− (n = 7) mice (B) and is expressed in pg/24 h. Prostaglandin E2 (PGE2) metabolite excretion is decreased by 50% in both male and female mPges1−/− mice. Thromboxane B2 (TxB2) and 6 keto-PGF1α are increased in female mPges1−/− versus female WT mice (P < 0.05). Prostanoid excretion is increased in female compared with male mice regardless of gender and genotype (P < 0.001 versus male mice for PGEM and TxB2; P < 0.01 for 6 keto-PGF1α). *P < 0.01 versus WT; †P < 0.05 versus WT; ‡P < 0.001 versus male mice; §P < 0.01 versus male mice.

**Expression of PGES and Cyclooxygenases in Kidneys of mPGE1-Null Mice**

To determine the effect of mPGE1 deletion on other components of the prostanoid synthetic pathway and to test for
potential gender effects on their expression in the kidney, we used real-time quantitative RT-PCR to compare mRNA abundance of COX-1, COX-2, mPGES1, mPGES2, and cPGES in groups of male and female mPges1+/+ and mPges1−/− mice. Among WT mice, there seemed to be a trend toward greater COX-1 mRNA abundance in WT female compared with male mice, but the difference did not reach statistical significance (Figure 2A). Conversely, levels of COX-2 mRNA in kidneys of WT female mice were approximately two-fold higher than those of WT male mice (P < 0.01), corresponding with the significantly higher urinary prostanoid excretion that we observed in female mice. Similarly, as shown in Figure 2B, levels of mPGES1 mRNA were also significantly higher in female compared with male mPges1+/+ mice (P < 0.01). There were no differences in expression of mPGES2 and cPGES between male and female WT mice.

As shown in Figure 2A, the absence of mPGES1 had no effect on COX-1 expression in male or female mice. By contrast, COX-2 mRNA expression was increased significantly in mPGES1−/− male mice compared with WT male controls (P = 0.01), and these levels were similar to those of mPGES1+/+ female mice. However, the absence of mPGES1 did not cause further augmentation of COX-2 expression in female mice as COX-2 mRNA expression was similar in female mPges1+/+ and mPges1−/− mice. As expected, mPGES1 expression was not detected in kidneys from male

Figure 2. Renal PGES and cyclooxygenase mRNA expression. Relative mRNA expression levels of mPGES1, mPGES2, and cPGES (A), as well as COX-1 and COX-2 (B) were assessed by real-time quantitative reverse transcriptase–PCR in kidneys of male and female mPges1−/− and WT control mice. Target mRNA expression levels were normalized to expression of 18S ribosomal RNA in the same tissue. Statistical significance was assessed by one-way ANOVA followed by Holm-Sidak post hoc test. *P < 0.01 versus male; #P < 0.01 versus WT.
or female mPges1\(^{-/-}\) mice (Figure 2B). Moreover, the absence of mPges1 had no effect on expression of the other putative PGES, mPGES2 or cPGES.

### mPges1 and Urinary Concentration

Previous studies have suggested that PGE\(_2\) may directly oppose the actions of vasopressin to promote water flux in the distal nephron, thereby modulating urinary concentrating mechanisms (4). To determine whether mPges1 might contribute to this response, we compared urinary osmolalities in mPges1\(^{-/-}\) and mPges1\(^{+/+}\) mice with free access to water and after 12-h of water deprivation. Baseline urine osmolalities were similar in mPges1\(^{-/-}\) (\(n = 8\)) and mPges1\(^{+/+}\) (\(n = 12\)) mice (1672 ± 180 versus 1876 ± 134 mOsm; NS). After 12 h of water deprivation, urine osmolalities increased significantly in both groups (\(P < 0.001\)), and the maximal urine osmolalities after thirsting were virtually identical in mPges1\(^{-/-}\) and mPges1\(^{+/+}\) mice (3431 ± 109 versus 3461 ± 73 mOsm; NS). Thus, the absence of mPges1 does not significantly alter the capacity of the renal concentrating mechanisms.

### Role of mPges1 in BP and Adaptation to Altered Dietary Sodium Intake

On the control (0.4% NaCl) diet, there were no differences in BP between male (117 ± 2 versus 118 ± 2 mmHg) and female (121 ± 1 versus 119 ± 2 mmHg) mPges1\(^{-/-}\) and mPges1\(^{+/+}\) mice, respectively. BP was not significantly affected by low-salt feeding in male (120 ± 2 versus 118 ± 2 mmHg) or female (120 ± 1 versus 121 ± 2 mmHg) mPges1\(^{-/-}\) and mPges1\(^{+/+}\) mice, respectively. Feeding the high-salt diet caused BP to increase significantly in all of the groups (\(P < 0.001\) by paired \(t\) test), but the magnitude of BP increases was similar in the WT and mPges1-deficient mice so that BP were similar at the end of the high-salt period in male (133 ± 3 versus 129 ± 2 mmHg) and female (132 ± 3 versus 134 ± 3 mmHg) mPges1\(^{-/-}\) and mPges1\(^{+/+}\) mice, respectively.

### Role of mPges1 in the Diuretic Response to Furosemide

To determine whether mPges1 might be the source of the enhanced PGE\(_2\) generation with furosemide and to define its contribution to facilitating the actions of furosemide in the kidney, we compared furosemide responses in groups of male and female mPges1\(^{-/-}\) and mPges1\(^{+/+}\) mice. As shown in Figure 3, administration of furosemide caused a brisk diuresis in male WT mice, with urine volume increasing from 0.31 ± 0.07 ml/4 h before treatment to 1.2 ± 0.07 ml/4 h after furosemide (\(P < 0.001\)). Urine sodium also increased significantly from 23 ± 7 to 147 ± 8 μmol/4 h (\(P < 0.001\)). Along with this diuresis and natriuresis, excretion of PGE\(_2\) metabolites increased in male WT mice from 73 ± 18 to 152 ± 28 pg/4 h (\(P < 0.05\)), whereas excretion of TxB\(_2\) and 6 keto-PGF\(_{1\alpha}\) was unaffected (Figure 4).

Furosemide had very similar effects in WT female mice (Figure 3). Urine volume increased from 0.26 ± 0.07 ml/4 h before treatment to 1.24 ± 0.1 ml/4 h with furosemide (\(P < 0.001\)). Sodium excretion also increased from 13.6 ± 4 to 132 ± 12 μmol/4 h (\(P < 0.001\); Figure 3). Although basal excretion of PGE\(_2\) was higher in the WT female compared with male mice, PGE\(_2\) excretion increased further after furosemide, from 89 ± 196 to 1664 ± 122 pg/4 h (\(P < 0.01\)). Similar to male mice, excretion of TxB\(_2\) and 6 keto-PGF\(_{1\alpha}\) was not altered by furosemide in female WT mice (Figure 4).

To determine whether furosemide effected expression of synthetic enzymes in the PGE\(_2\) pathway, we compared mRNA levels for mPges1 in WT DBA/1 mice 4 h after administration of vehicle or furosemide. Levels of mPges1 mRNA were similar in vehicle- (1.48 ± 0.22) and furosemide-treated mice (1.32 ± 0.10), suggesting that furosemide had no effect on mPges1 expression during the interval tested.

As shown in Figure 3, compared with male mPges1\(^{-/-}\) mice, response to furosemide was significantly attenuated in the male mPges1\(^{+/+}\) mice. Urine flow (0.95 ± 0.05 versus 1.2 ± 0.07 ml/4 h; \(P < 0.05\)) and sodium excretion (117 ± 6 versus 147 ± 8 μmol/4 h; \(P < 0.01\)) after furosemide injection were approximately 20% lower in male mPges1-deficient mice than in WT controls. However, both urine flow and sodium excretion were increased significantly compared with baseline conditions (\(P < 0.001\)). Furthermore, unlike in WT male mice, furosemide had no effect on PGE\(_2\) metabolite excretion in the male mPges1\(^{-/-}\) mice (73 ± 18 versus 74 ± 10 pg/4 h). Accordingly, as shown in Figure 4, excretion of PGE\(_2\) metabolites after administration of furosemide was significantly lower in the male mPges1-deficient mice (74 ± 10 pg/4 h) than in male, WT controls (152 ± 28 pg/4 h; \(P < 0.05\)).

Unlike the male mPges1\(^{-/-}\) mice, the extent of furosemide-induced diuresis was not altered in female mPges1\(^{-/-}\) mice. As shown in Figure 3, urine flow (0.28 ± 0.03 versus 1.22 ± 0.07 ml/4 h; \(P < 0.001\)) and sodium excretion (18 ± 7 versus 145 ± 11 μmol/4 h; \(P < 0.001\)) increased significantly. Moreover, after furosemide, urine flow (1.22 ± 0.07 versus 1.24 ± 0.1 ml/4 h) and sodium excretion (145 ± 11 versus 132 ± 12 μmol/4 h) were virtually identical in female mPges1\(^{-/-}\) and mPges1\(^{+/+}\) mice. As shown in Figure 4, despite this preservation of furosemide diuresis in the mPges1\(^{-/-}\) female mice, PGE\(_2\) metabolite excretion was modestly but not significantly augmented after furosemide.
Prostanoid excretion, the levels of PGE2 excretion after furosemide-stimulated prostanoid excretion (575 ± 122 pg/4 h; P < 0.05) and 6 keto-PGF1α (881 ± 166 versus 4397 ± 637 pg/4 h; P < 0.01) increased after furosemide in the female mPGES1-deficient mice (Figure 4).

To explore further the relationship between furosemide-induced diuresis and prostanoid generation in female mice, we examined the consequences of a more complete inhibition of prostanoids by pretreating mice with the COX inhibitor diclofenac (10 mg/kg). Pretreatment of female WT mice with diclofenac caused a significant attenuation of furosemide on prostanoid generation and diuresis in WT female mice (Figure 5, A and B). Urine flow was decreased by approximately 35%, from 1.38 ± 0.13 ml/4 h without pretreatment to 0.89 ± 0.09 ml/4 h with diclofenac (P < 0.05). Sodium excretion was similarly reduced from 145 ± 13 to 108 ± 12 μmol/4 h (P < 0.05) with the COX inhibitor. As shown in Figure 5C, along with its effects on the diuretic response, diclofenac caused a broad inhibition of furosemide-stimulated prostanoid excretion (575 ± 252 versus 2044 ± 252 pg/4 h for PGE2 [P < 0.001]; 237 ± 42 versus 561 ± 123 pg/4 h for TxB2 [P < 0.05]; 248 ± 36 versus 511 ± 126 pg/4 h for 6 keto-PGF1α [P < 0.05]).

**Furosemide-Stimulated Renin Expression**

PGE2 is a potent secretagogue for renin, where it operates as part of a signaling pathway that includes COX-2. In the macula densa, reduced solute flux through the Na-K-2Cl co-transporter (NKCC2) regulates the PGE2-dependent signal for renin (28). To examine the role of mPGES1 in this pathway, we compared the effects of chronic NKCC2 blockade with furosemide on renin stimulation in mPges1+/− and mPges1−/− mice. There was no significant difference in renin expression between untreated mPges1+/− (1.03 ± 0.17) and mPges1−/− mice (0.71 ± 0.26). Administration of furosemide to WT mice for 5 d caused a marked stimulation of renin expression (5.68 ± 0.56; P = 0.002). Furosemide caused a similar stimulation of renin in the mPges1-deficient mice (6.44 ± 1.02; P = 0.006). However, the extent of furosemide-stimulated renin expression was not significantly different between the mPges1+/− and mPges1−/− mice, suggesting that mPGES1 does not have a unique, nonredundant role to stimulate renin mRNA expression via this mechanism.

**Discussion**

PGE2 is a key product of the COX pathway of arachidonic acid metabolism. This lipid mediator has diverse actions that include cytoprotective functions in the gastrointestinal tract, regulating smooth muscle tone in airways and vasculature, modulating the immune system, and triggering febrile responses in the brain (29–31). PGE2 is a major arachidonic acid metabolite in the kidney, where it may affect control of renal blood flow, sodium excretion, and BP (1). It is produced by a series of three enzymatic reactions: release of arachidonic acid from membrane phospholipids by the actions of phospholipases, conversion of arachidonic acid to unstable endoperoxide intermediates by COX-1 and -2, and, finally, isomerization of PGH2 to PGE2 by terminal PGES. The temporal and spatial properties of PGE2 synthesis are determined by the regulated expression of each of the enzymes involved in this cascade. Of
the several putative PGES that have been described, mPGES1 is the best characterized, and a role for mPGES1 in inflammation and febrile responses has been clearly demonstrated (21,32,33). Although mPGES1 is expressed in the kidney (17–19,34), its contribution to PGE2 synthesis in the kidney and to the consequent regulation renal functions has not been characterized previously. Relative to other COX products, PGE2 is generated in substantial quantities by the kidney (1). Major sites of PGE2 generation by the kidney are renal medullary interstitial cells and collecting duct (1,35–37). Studies using in situ hybridization and immunocytochemistry have documented mPGES1 expression in the distal nephron from the connecting tubule through the cortical and medullary collecting duct (17–19,34). In this region, the distribution of mPGES1 parallels expression of COX-1 (18,19).

Our studies suggest that mPGES1 makes a nonredundant contribution to renal PGE2 production, because urinary PGE met abolite excretion is reduced by approximately 50% in mPGES1-deficient animals in the basal state. Our findings are consistent with another recent article that showed a marked reduction of urinary PGE2 metabolite excretion, measured by gas chromatography–mass spectrometry, in mPGES1-deficient mice compared with controls (38). Although the magnitude of the impact of mPGES1 deficiency was similar across genders, in mPGes1−/− female but not mPGes1−/− male mice, there was a proportional increase in excretion of other prostanoids, including PGI2 (prostacyclin). This may result from shunting of endoperoxides toward other prostanoid synthetic pathways. This phenomenon has been observed in other circumstances when prostanoid synthetic enzymes are genetically deleted (39) or pharmacologically inhibited (40). Because PGL2 and PGE2 have some redundant functions (8), this increase in PGL2 generation might act as a compensatory mechanism to ameliorate the physiologic consequences of mPGES1 deficiency. Similar reductions in basal PGE2 generation with shunting have been reported in other tissue preparations from mPges1−/− mice (41,42). We also found prominent differences in renal prostanoid generation between male and female mice in our studies with basal levels of prostanoid excretion that are almost fourfold higher in female than male mice. These were accompanied by enhanced expression of mRNA for synthetic enzymes, including COX-2 and mPGES1 in kidneys of female compared with male mice.

Although our data assign a unique role for mPGES1 in renal PGE2 generation, we find significant residual levels of PGE2 production in the absence of mPGES1. Moreover, we and others have previously shown that ductus arteriosus closure is dependent on activation of the EP4 receptor for PGE2 (43,44), yet mPges1-deficient mice undergo normal closure of the ductus arteriosus (21,32). Taken together, these data indicate that there are alternative, mPGES1-independent pathways for PGE2 synthesis in the intact animal. The nature of these pathways is not clear, but our unpublished data suggest that another puta-
tive PGE synthase, mPGES2, does not make a significant contribution to PGE2 generation in vivo (Jania et al., submitted).

Among its wide range of physiologic actions, PGE2 has a number of effects that could affect BP homeostasis. It is a potent vasodilator in both the peripheral (45) and renal vasculature (46). Moreover, PGE2 has potent natriuretic effects in the kidney through direct epithelial effects on salt and water transport and as a consequence of its hemodynamic actions to increase renal blood flow (3,8). Accordingly, it has been suggested that inhibition of PGE2 could explain the actions of NSAID and COX-2 inhibitors to cause edema and hypertension. However, PGE2 is a potent secretogogue for renin, and the consequences of activation of the renin-angiotensin system by PGE2 are in direct opposition to its effects on renal vasculature and epithelia that lead to vasodilation and natriuresis. However, our data do not support a role for mPGES1 in furosemide-stimulated renin release. These findings are consistent with previous studies that suggested that PGI2, acting through the I-prostanoid (IP) receptor, may be the critical pathway mediating stimulation of renin with NKCC2 blockade (47).

The role of individual pathways for PGE2 synthesis in regulating BP is not known. Our data suggest that mPGES1 does not play a major role in BP homeostasis, because BP was virtually identical in mPges1+/+ and mPges1−/− mice across a range of dietary sodium intakes. This finding is consistent with the recent report by Cheng et al. (38), who also found no difference in BP between mPges1−/− and WT control mice on either normal- or high-salt diet. Furthermore, our previous studies implicated alterations in PGI2 in NSAID-associated hypertension (48). Nonetheless, it is still possible that mPGES1 might have played a role in modulating BP and vascular tone on a different genetic background or in models of hypertension.

Among its actions in the kidney, PGE2 may influence water permeability in the distal nephron by opposing the actions of the Gs-linked vasopressin receptor (49). Because of its linkage to inhibitory G-protein (Gi) and its high levels of expression in renal medulla, the EP3 receptor was suggested as a candidate to mediate the actions of PGE2 in opposing vasopressin-stimulated water flow (50), yet in a previous study, we found only subtle changes in urinary concentrating and diluting mechanisms in EP3-deficient mice (51). In these studies, we find that the absence of mPGES1 has no effect on urinary concentrating capacity. Accordingly, insofar as PGE2 may modulate vasopressin actions in the intact animal, mPGES1 is not absolutely required. It is possible that the residual level of PGE2 generated in kidneys of mPGES1 mice is sufficient to fulfill this function. Alternatively, the capacity of PGE2 to influence vasopressin actions in vivo may be less robust than suggested by studies in cell culture or isolated perfused nephron segments (50,52). However, alterations in urinary concentrating capacity did not become apparent in cytosolic phospholipase A2 (cPLA2)-deficient mice until >320 d of age (24), and our studies were carried out in mice that were <6 mo of age.

Evidence from clinical studies indicates a contribution of PGE2 to the sodium excretion that is induced by furosemide (6,53). For example, in healthy humans, a correlation was found between sodium excretion and the furosemide-induced increment in PGE2 (53). Our studies indicate that mPGES1 is the primary source of furosemide-stimulated PGE2 release, because the enhancement of PGE2 generation after furosemide is virtually abolished in mPGES1-deficient mice. Blockade of NKCC2 by furosemide stimulates prostanooid generation in part via upregulation of COX-2 (54). Our data suggest that mPGES1 is also linked to this pathway and, at least in males, directly contributes to diuresis and natriuresis. However, we find no effect of furosemide on mPges1 expression in the kidney, suggesting that stimulation of prostanooid excretion by furosemide occurs at a level upstream of mPGES1.

mPGES1 is also essential for furosemide-stimulated PGE2 generation in female mice. However, this augmentation of PGE2 generation is not required for the full diuretic response in female mice. Because COX inhibition reduces the extent of furosemide diuresis in WT female mice, there are at least two possible explanations for the preserved furosemide response in female mPGES1-deficient mice. First, despite their failure to augment PGE2 excretion with furosemide, the levels of PGE2 excretion in female mPGES1-deficient mice exceed those seen in male WT mice after furosemide administration. Thus, there may be a threshold level of PGE2 excretion required to facilitate diuresis with furosemide, and this level may be exceeded even in the basal state in mPGES1-deficient female mice. Alternatively, the increase in PGI2 generation that is seen in the female mPGES1-deficient mice may act as a compensatory mechanism to blunt the impact of their failure to augment PGE2 generation after furosemide.

Until the recent development of mPGES1-deficient mouse lines (21,32), very little was known about the in vivo functions of the different forms of PGES. Studies using mPges1−/− mice indicate that mPGES1 is responsible for the late phase of PGE2 synthesis during inflammation via a pathway that depends on COX-2 (21,32). Febrile responses to LPS are also mediated by mPGES1 (33), and mPGES1 is the source of PGE2 that facilitates acute inflammatory pain (21). Expression of mPGES1 is markedly upregulated in synovial tissues in arthritis (12,55), contributing to the pathogenesis of joint inflammation and swelling (21). Taken together, these observations suggest that inhibition of mPGES1 might be useful for the treatment of inflammation and pain, providing a more precise therapeutic target than NSAID or COX-2 inhibitors. Nonetheless, the utility of this approach would depend on whether some of the untoward effects that are associated with global COX inhibition, such as gastrointestinal toxicity, hypertension, and adverse renal effects, would be avoided by specific mPGES1 inhibition. Our findings predict that there would be a preservation of a residual capacity for PGE2 generation in the kidney with mPGES1 inhibition that might protect against renal complications of COX inhibition, such as hypertension. However, some resistance to loop diuretics might be expected.

Acknowledgments

This work was supported by National Institutes of Health grant DK069896 and by funding from the Medical Research Service of the Veterans Administration.
We acknowledge outstanding administrative support from Norma Turner.

Disclosures
None.

References


