Abstract—Prostaglandin (PG)E₂ has multiple actions that may affect blood pressure. It is synthesized from arachidonic acid by the sequential actions of phospholipases, cyclooxygenases, and PGE synthases. Although microsomal PGE synthase (mPGES)1 is the only genetically verified PGE synthase, results of previous studies examining the consequences of mPGES1 deficiency on blood pressure (BP) are conflicting. To determine whether genetic background modifies the impact of mPGES1 on BP, we generated mPGES1⁻/⁻ mice on 2 distinct inbred backgrounds, DBA/1lacJ and 129/SvEv. On the DBA/1 background, baseline BP was similar between wild-type (WT) and mPGES1⁻/⁻ mice. By contrast, on the 129 background, baseline BPs were significantly higher in mPGES1⁻/⁻ animals than WT controls. During angiotensin II infusion, the DBA/1 mPGES1⁻/⁻ and WT mice developed mild hypertension of similar magnitude, whereas 129-mPGES1⁻/⁻ mice developed more severe hypertension than WT controls. DBA/1 animals developed only minimal albuminuria in response to angiotensin II infusion. By contrast, WT 129 mice had significantly higher levels of albumin excretion than WT DBA/1 and the extent of albuminuria was further augmented in 129 mPGES1⁻/⁻ animals. In WT mice of both strains, the increase in urinary excretion of PGE₂ with angiotensin II was attenuated in mPGES1⁻/⁻ animals. Urinary excretion of thromboxane was unaffected by angiotensin II in the DBA/1 lines but increased more than 4-fold in 129 mPGES1⁻/⁻ mice. These data indicate that genetic background significantly modifies the BP response to mPGES1 deficiency. Exaggerated production of thromboxane may contribute to the robust hypertension and albuminuria in 129 mPGES1-deficient mice. (Hypertension. 2010;55[part 2]:531-538.)

Key Words: prostanooids ■ PGE synthase ■ blood pressure ■ strain ■ hypertension
cardiovascular consequences such as hypertension and coronary thrombosis associated with COX inhibitors might be avoided by specific mPGES1 inhibitors.

The effects of mPGES1 deficiency on blood pressure and atherosclerosis have also been examined in several previous studies. For example, in a mouse model of atherosclerosis, the absence of mPGES1 was associated with attenuated atherogenesis. On the other hand, studies of blood pressure regulation in mPGES1-deficient mice have yielded conflicting results, wherein mPGES1-deficient mice have been reported to have normal or reduced baseline blood pressures with normal or exaggerated responses to high-salt feeding. Moreover, one group has reported that these animals have increased susceptibility to angiotensin (Ang) II–dependent hypertension. The genetic background of these various mPGES1-deficient mouse lines were all different. Because genetic background can have a marked influence on the phenotypes of genetically modified mice, we considered the possibility that the variable blood pressure responses observed in studies of mPGES1−/− mice might be related to effects of strain-specific genetic modifiers. To address this issue, we generated mPGES1−/− mice on 2 distinct inbred backgrounds, DBA/1lacJ (DBA/1) and 129/SvEv (129) and compared blood pressure at baseline and during Ang II–dependent hypertension. We find strong effects of genetic background to influence the consequences of mPGES1 deficiency on blood pressure, albuminuria, and the generation of other vasoactive prostanoids.

Methods

Animals
Production of inbred DBA/1 mice with targeted disruption of the mPGES1 (Pges1) gene using embryonic stem cells derived from DBA/1lacJ inbred mice has been described previously. DBA/1lacJ and 129/SvEv mice were generated by backcrossing the mPGES1 mutation onto the 129/SvEv genetic background for more than eight generations. Inbred DBA/1lacJ and 129/SvEv mice were considered significant.

Statistical Analyses
All data are presented as means ± SEM. Differences between groups were analyzed by unpaired t test or 2-way ANOVA, followed by Bonferroni post hoc test, as indicated, using GraphPad Prism software version 4.00 (GraphPad Software, San Diego, Calif). Differences within groups, before and after Ang II infusion, were analyzed by paired t test. A probability value of <0.05 was considered significant.

Results
We first measured blood pressures in the different mouse lines using radiotelemetry. At baseline, blood pressures in the wild-type (WT) 129 mice (113 ± 1 mm Hg) were significantly higher than the WT DBA/1 animals (105 ± 1 mm Hg; P < 0.001). As shown in Figure 1, there were no differences in blood pressure between DBA/1 WT and mPGES1−/− mice.
Angiotensin II–dependent hypertension in DBA/1lac/J and 129/SvEv mice in the presence and absence of mPGES1. Ang II (1000 ng/kg per minute) was infused for 3 weeks by osmotic minipump while blood pressure was measured simultaneously by radiotelemetry. A, Time course of Ang II–induced hypertension in WT and mPGES1-deficient mice on the DBA/1lacJ and 129/SvEv genetic backgrounds. B, Group means for 3 weeks of Ang II infusion in DBA/1 (WT, n=8; mPGES1−/−, n=8) and 129/SvEv (WT, n=8; mPGES1−/−, n=5) mice. *P<0.05 vs WT; #P<0.05 vs DBA/1 by 2-way ANOVA with Bonferroni post test.

It has been suggested that stimulation of PGE2 synthesis may attenuate blood pressure elevation in various hypertensive states.33 Therefore, we next investigated the consequences of mPGES1 deficiency on Ang II–induced hypertension. As shown in Figure 2A, continuous infusion of Ang II increased blood pressure by 30 to 40 mm Hg over the first 3 to 4 days in the WT DBA/1 mice, followed by a waning of the blood pressure level over the next week, achieving a new, more modestly elevated level by the end of the infusion period. The pattern and magnitude of the hypertensive response to chronic Ang II was virtually identical in the DBA/1 mice lacking mPGES1, and, thus, the average of the mean arterial pressures for the 3 weeks of Ang II infusion were similar in the DBA/1 WT (123±2 mm Hg) and mPGES1-deficient mice (124±3 mm Hg; Figure 2B). As shown in Figure 2A and 2B, the extent of the blood pressure elevation in the WT DBA/1 mice was significantly greater than the WT DBA/1 animals (145±4 versus 123±2 mm Hg; P<0.001). Moreover, the marked elevation in blood pressure in the 129-WT group was sustained throughout the 3 weeks of infusion (Figure 2A). The 129-mPGES1−/− group developed hypertension that was significantly more severe compared to their 129-WT littermates (160±4 versus 145±4 mm Hg, P=0.01; Figure 2B).

As shown in Figure 3 (left), levels of renin mRNA expression were significantly higher in kidneys from mPGES1−/− DBA/1 than their WT controls at baseline (172±10 versus 100±13% of WT, P<0.05). This baseline difference was not seen in the 129 mice (100±11 versus 92±12% of WT). Renin mRNA expression was suppressed significantly and to similar levels in both the DBA/1 (37±10 versus 57±16% of WT baseline; WT P<0.05 versus baseline) and 129 mice (44±7 versus 36±8% of WT baseline; P<0.01 versus baseline) during Ang II infusion.

To determine whether mPGES1 affects kidney damage induced by Ang II, we measured urinary albumin excretion at baseline and after 3 weeks of Ang II infusion. Basal levels of albumin excretion were similar in DBA and 129 WT mice (50±18 versus 15±4 µg/24 hours) and were unaffected by mPGES1 deletion in both strains of mice (DBA: 37±7; 129: 17±5 µg/24 hours) (data not shown). With Ang II infusion, urinary albumin excretion increased only modestly in the WT DBA/1 animals (103±22 µg/24 hours) and levels of albuminuria were basically unaffected by the absence of mPGES1 in the DBA/1-mPGES1−/− group (114±23 µg/24 hours; Figure 4).

Figure 3. Renin mRNA expression in kidneys of DBA/1 and 129/SvEv mice. Whole kidney cDNA was used for real-time quantitative RT-PCR analysis of renin mRNA levels at baseline and after 3 weeks of Ang II infusion. Data are expressed as percentages of DBA/1 and 129 WT baseline. *P<0.05 vs baseline; #P<0.05 vs WT by 2-way ANOVA with Bonferroni post test.
By contrast, Ang II caused marked proteinuria in the WT 129 mice such that their levels of albumin excretion (514±106 µg/24 hours) were significantly higher than the WT DBA/1 group (P=0.002), as shown in Figure 4. The extent of albuminuria was further augmented in the 129 mPGES1−/− animals (1167±164 µg/24 hour; P=0.003 versus WT).

To examine whether the absence of mPGES1 affected prostanooid metabolism and whether this might be affected by genetic background, we measured urinary prostanooid excretion in the various lines at baseline and during Ang II infusion. At baseline, urinary PGE2 excretion was similar in DBA and 129 WT mice (813±120 versus 808±137 pg/mg creatinine, Figure 5). Mice lacking mPGES1 had reduced baseline PGE2 excretion compared to WT, although the difference did not reach statistical significance in 129 animals (DBA: 398±87 pg/mg creatinine, P=0.01; 129: 550±119 pg/mg creatinine, P=0.09; Figure 5). Chronic infusion of Ang II caused significant increases in urinary excretion of PGE2 metabolite in WT mice of both strains (DBA: 1968±498 pg/mg creatinine, P<0.01 versus baseline; 129: 3962±1022 pg/mg creatinine, P<0.001 versus baseline; Figure 5). The levels of PGE2 metabolite in urine during Ang II infusion tended to be higher in the WT 129 compared to WT DBA/1; however, this difference did not achieve statistical significance. As shown in Figure 5, the increased PGE2 excretion during Ang II–dependent hypertension was attenuated in mPGES1−/− animals of both strains as compared to WT controls, although, again, the difference between 129-WT and mPGES1−/− animals was not statistically significant (DBA: 781±187 [mPGES1−/−] versus 1968±498 [WT] pg/mg creatinine, P=0.032; 129: 1508±585 [mPGES1−/−] versus 3962±1022 [WT] pg/mg creatinine, P=0.06).

We next measured urinary excretion of TxB2, the stable metabolite of the potent vasoconstrictor prostanooid TxA2 (Figure 6, top graphs), which has been implicated in the pathogenesis of Ang II–dependent hypertension.34–36 At baseline, there were no differences in excretion of urinary TxB2 between WT DBA/1 and 129 mice (1542±391 versus 1976±529 pg/mg creatinine). On the DBA/1 background, absence of mPGES1 has no effect on TxB2 excretion at baseline (1376±232 versus 1542±391 pg/mg creatinine, P=NS), whereas urinary TxB2 was higher in 129-mPGES1−/− mice compared to the 129 WT controls, although the difference was not statistically significant (3402±796 versus 1976±529: P=0.074). As shown in Figure 6 (top graphs), chronic infusion of Ang II had no effect on urinary TxB2 excretion in the DBA lines (WT 1542±391 to 1319±172 pg/mg creatinine, P=NS; mPGES1−/− 1376±232 to 1488±300 pg/mg creatinine, P=NS). In marked contrast, there were significant increases in urinary TxB2 excretion with Ang II infusion by 2-fold in 129 WT (1976±529 to 4043±1077 pg/mg creatinine, P=0.047) and more than 4-fold in 129 mPGES1−/− mice (3402±796 to 14235±3948 pg/mg creatinine, P=0.002) (Figure 6, top right).

We measured urinary excretion of 6-keto-PGF1α, the stable metabolite of prostacyclin, a potent vasodilator and anti-thrombotic prostanooid (Figure 6, bottom). At baseline, excretion of urinary 6-keto-PGF1α was very similar in all 4 experimental groups, and there was no difference in excretion of the PGJ2 metabolite between WT and mPGES1-deficient mice on the DBA/1 (5350±1264 versus 6948±924 pg/mg creatinine, P=NS) or 129 backgrounds (5223±1291 versus 5218±1153, P=NS). As shown in Figure 6, urinary 6-keto-PGF1α excretion in the DBA lines was virtually unaffected by chronic infusion of Ang II (WT 5350±1264 to 4375±670 pg/mg creatinine, P=NS; mPGES1−/− 6948±924 to 6145±1108 pg/mg creatinine, P=NS). By contrast, there were significant increases in urinary 6-keto-PGF1α excretion with Ang II infusion by more than 3-fold in 129 WT (5223±1291 to 17836±4088 pg/mg creatinine, P<0.01) and
by nearly 7-fold in 129 mPGES1<sup>−/−</sup> mice (5218±1153 to 35517±5463 pg/mg creatinine, P<0.001).

We next measured mRNA levels for the COX enzymes COX-1 and -2 (Figure 7). At baseline, the relative expression of COX-1 in the kidney was not significantly different between WT and mPGES1-deficient mice from both strains (100±13 versus 130±17% for DBA/1 and 100±9 versus 73±5% of WT for 129). Infusion of Ang II did not affect COX-1 mRNA abundance in the DBA/1 mice (WT 111±26 versus mPGES1<sup>−/−</sup> 116±22% of WT baseline). In contrast, COX-1 mRNA levels increased with Ang II infusion by ≈50% in both 129 WT (150±21% of WT baseline, P=NS) and mPGES1<sup>−/−</sup> animals (146±21% of WT baseline, P<0.05), although this increase was statistically significant only in the mPGES1<sup>−/−</sup> group. As shown in Figure 7, baseline COX-2 expression was 2-fold higher in kidneys of DBA/1 mPGES1<sup>−/−</sup> animals than their WT controls (100±18 versus 204±38% of WT; P<0.05), but there was no difference in COX-2 mRNA levels in the 129 mPGES1<sup>−/−</sup> mice at baseline (100±29 versus 147±6% of WT; P=0.061). Ang II infusion did not significantly alter COX-2 mRNA abundance in either group of DBA/1 mice (WT 163±18 versus mPGES1<sup>−/−</sup> 184±39% of WT baseline), whereas COX-2 mRNA levels increased 2-fold with Ang II infusion in both 129 WT (197±43% of WT baseline, P=NS) and mPGES1<sup>−/−</sup> animals (279±36% of WT baseline, P<0.05), but this increase was statistically significant only in the 129-mPGES1<sup>−/−</sup> group.

**Discussion**

A role for PGE_2_ in blood pressure homeostasis was first suggested by studies documenting its vasodilator actions in the systemic circulation. Infusion of PGE_2_ into the kidney commonly causes renal vasodilation. Moreover, relative to other COX products, PGE_2_ is generated in substantial quantities by the kidney, where it is has potent natriuretic effects. The major sites of PGE_2_ generation in the kidney are renal medullary interstitial cells and collecting ducts. 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 blood pressure homeostasis.

To examine the contributions of PGE_2_ to blood pressure homeostasis, mice lacking mPGES1 have been used. We previously found that the absence of mPGES1 in mice on an inbred DBA/1 background had no effect on blood pressures during feeding of normal (0.4% NaCl) or high (6% NaCl) salt diets. These findings were similar to the report from the FitzGerald group demonstrating normal blood pressures in mice lacking mPGES1. By contrast, Yang and colleagues found that mPGES1-deficient mice developed hypertension with high salt feeding. Moreover, they also reported that mPGES1-deficient mice were more susceptible to hypertension caused by chronic infusion of Ang II, manifesting exaggerated oxidative stress in this setting. On the other hand, subsequent work by FitzGerald and colleagues showed that deletion of mPGES1 in low-density lipoprotein receptor-deficient mice does not affect their hypertensive response to chronic infusions of Ang II but protects them from aortic aneurysm formation and significantly reduces oxidative stress. The reasons for the differing outcomes of these studies of mPGES1-deficient mice are not clear. Because the strain combinations are different in each of the mPGES1-deficient lines from these studies, the effects of genetic
background to influence the mPGES1 phenotype may be one factor explaining the different phenotypes.

To evaluate the potential for strain-specific factors to modify the phenotype of mPGES1 deficiency, we generated mice lacking mPGES1 on 2 distinct genetic backgrounds: DBA/1lacJ and 129/SvEv. DBA/1 mice are susceptible to the development of collagen-induced arthritis, and 129 mice are salt-sensitive with enhanced susceptibility to kidney injury. We found that WT 129/SvEv mice have higher baseline blood pressure than WT DBA/1lacJ animals. Although deletion of mPGES1 had no effect on blood pressure on the DBA/1 background, blood pressures were significantly higher in mPGES1-deficient 129 mice compared to the 129 WT controls. Thus, genetic background significantly modifies the consequences of mPGES1 deficiency on baseline blood pressure.

Along with the effects on baseline blood pressure, the 129 background also proved more susceptible to the development of hypertension. Whereas the mPGES1-deficient mice on the DBA/1 background had blood pressure responses to chronic Ang II infusion that were virtually identical to the DBA/1 WT group, the severity of hypertension was substantially augmented in the mPGES1-deficient 129 mice. Because the extent of blood pressure elevation was also more severe in the 129-mPGES1−/−, it is not clear whether the worsening proteinuria was attributable to more severe hypertension or reflected a vulnerability to kidney damage caused by the absence of mPGES1. By contrast, DBA/1 WT mice developed only modest albuminuria during Ang II infusion, and this was not augmented in the DBA/1-mPGES1−/− animals. These findings show that actions of mPGES1 to protect against kidney injury are also conditioned by genetic background.

PGE2 synthesis is stimulated during hemodynamic stress, and it has been suggested that increased levels of PGE2 may be a compensatory response to attenuate the development of hypertension. Chronic infusion of Ang II stimulated increased urinary excretion of PGE metabolite in WT mice of both DBA/1 and 129 strains. Elimination of mPGES1 significantly attenuated PGE2 metabolite excretion by a similar amount (∼60%) in mPGES1-deficient mice on both DBA and 129 backgrounds (Figure 5). Thus, mPGES1 plays a significant role to increase PGE2 production in response to

Figure 7. Expression of COX isoforms COX-1 and COX-2 in mouse kidneys. Total RNA was isolated from kidneys and levels of mRNA for COX-1 (top graphs) and COX-2 (bottom graphs) were measured by real-time RT-PCR at baseline and after 3 weeks of Ang II infusion in DBA/1 (A and C) and 129/SvEv (B and D) mice. *P<0.05 vs baseline by 2-way ANOVA with Bonferroni post test; #P<0.05 vs WT by 2-way ANOVA with Bonferroni post test.

Enhanced hypertension in the 129 animals was also accompanied by exaggerated kidney injury as reflected by increased albumin excretion during Ang II infusion. This was apparent in the 129 WT mice compared to the WT DBA/1 group, consistent with enhanced susceptibility to kidney injury that has been observed in this strain. Furthermore, albuminuria induced by Ang II infusion was dramatically enhanced in the mPGES1-deficient 129 mice. Because the extent of blood pressure elevation was also more severe in the 129-mPGES1−/−, it is not clear whether the worsening proteinuria was attributable to more severe hypertension or reflected a vulnerability to kidney damage caused by the absence of mPGES1. By contrast, DBA/1 WT mice developed only modest albuminuria during Ang II infusion, and this was not increased in the DBA/1-mPGES1−/− animals. These findings show that actions of mPGES1 to protect against kidney injury are also conditioned by genetic background.
Ang II. However, there is significant residual production of PGE\(_2\) in both lines of mPGE\(_1^{-/-}\) mice, suggesting that there are robust pathways for PGE\(_2\) synthesis in vivo that do not require mPGE\(_1\). Despite a similar diminution of PGE\(_2\) excretion and higher absolute levels of urinary PGE\(_2\) metabolite with Ang II infusion in mPGE\(_1^{-/-}\) animals on the 129 background, hypertension was significantly more severe in the 129 compared to DBA/1 mice. Accordingly, the different blood pressure responses cannot be explained by differences in PGE\(_2\) metabolism.

Altered generation of other prostanoids, such as the potent vasoconstrictor TxA\(_2\), might explain the variable blood pressure responses that we observed. At baseline, urinary excretion of TxB\(_2\), the stable metabolite of TxA\(_2\), was similar in all of the DBA/1 and 129 lines. Ang II had no effect on urinary TxB\(_2\) levels in DBA/1 mice but stimulated urinary TxB\(_2\) excretion in the 129 mice that was more marked in the 129-mPGE\(_1\)-deficient mice to levels that were much more pronounced than any of the other experimental groups. The general patterns for urinary excretion patterns of 6-keto-PGF\(_1\alpha\), the major metabolite of PGI\(_2\), resembled those for thromboxane. The more marked stimulation of thromboxane and prostacyclin with Ang II in the 129 strain mice was mirrored by increased expression of both COX isoforms.

The precise cause of enhanced susceptibility to hypertension in the 129-mPGE\(_1^{-/-}\) mice is not completely clear from our studies. Although this cannot be explained by altered renin responses, differences in COX expression and prostanoid production in response to Ang II were quite marked between the 129 and DBA/1 lines. In particular, we speculate that augmented generation of thromboxane may contribute to the severity of hypertension in 129 mPGE\(_1^{-/-}\) mice because previous studies by our group using thromboxane receptor–deficient mouse lines have shown that enhanced generation of TxA\(_2\) contributes to blood pressure elevation and end-organ damage in Ang II–dependent hypertension. However, the physiological consequences of altered prostaglandin generation are complex and are likely determined by the balance of vasoconstrictor and vasodilator prostanoids at key tissue sites. In this regard, the ratio of TxB\(_2\)/PGE\(_2\) is increased more than 9-fold in the 129-mPges1\(^{-/-}\) mice compared to only 2-fold in the 129 WT controls. This indicates a tendency toward enhanced thromboxane generation in the mPGE\(_1\)-deficient mice on the 129 strain mice that could contribute to their susceptibility to develop hypertension.

Expression of mPGE\(_1\) is enhanced in inflammatory states, and this is likely responsible for the increased production of PGE\(_2\) that accompanies inflammation. Studies in mice lacking individual E-prostanoid receptor isoforms suggest that PGE\(_2\) is responsible for inflammatory pain and fever. It is perhaps not surprising that genetic deletion of mPGE\(_1\) recapitulates many of the beneficial antiinflammatory actions of NSAIDs. Accordingly, it has been suggested that small molecule inhibitors of mPGE\(_1\) might be useful antiinflammatory agents encompassing the beneficial effects of NSAIDs without the associated cardiovascular hazard. With regard to the propensity to cause hypertension, our study suggests that the impact of mPGE\(_1\) blockade on blood pressure may be influenced by genetic background and associated stimulation of TxA\(_2\) production.

**Perspectives**

mPGE\(_1\) inhibitors have been proposed as a potential new class of NSAIDs with fewer cardiovascular side effects compared to traditional NSAIDs or selective COX-2 inhibitors. Our data suggest that inhibiting mPGE\(_1\) may produce variable cardiovascular effects depending on genetic and environmental factors. Identification of genetic variables that influence the renal and cardiovascular response to mPGE\(_1\) inhibition may facilitate safe and effective therapies targeting this enzyme.

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**Disclosures**

None.

**References**


The Impact of Microsomal Prostaglandin E Synthase 1 on Blood Pressure Is Determined by Genetic Background

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