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THE IMPACT OF MICROSOMAL PROSTAGLANDIN E SYNTHASE 1 (mPGES1) ON BLOOD PRESSURE IS DETERMINED BY GENETIC BACKGROUND

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Abstract

Prostaglandin (PG) E_2 has multiple actions that may affect blood pressure. It is synthesized from arachidonic acid by the sequential actions of phospholipases, cyclooxygenases, and PGE synthases. While microsomal PGE synthase 1 (mPGES1) 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 two 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, while 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 PGE_2 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.

Keywords

prostanoids; PGE synthase; blood pressure; strain; hypertension

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CONFLICTS OF INTEREST/DISCLOSURES

None.

INTRODUCTION

Prostaglandin (PG) E_2 is a key product of the cyclooxygenase (COX) pathway of arachidonic acid metabolism. This lipid mediator has diverse actions that include cytoprotective functions in the GI tract, regulating smooth muscle tone in airways and vasculature, modulating the immune system, and triggering febrile responses in the brain[1-3]. In the kidney, PGE_2 is one of the most abundant metabolites of arachidonic acid and thus has a significant capacity to influence renal blood flow, sodium excretion, and regulation of blood pressure [4]. These actions may be through direct effects on renal vascular tone [5, 6] and epithelial function [7, 8], and indirectly through its actions to trigger renin release at the macula densa [9-11].

PGE_2 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 cyclooxygenases (COX-1 and –2), and finally, isomerization of PGH_2 to PGE_2 by terminal PGE synthases (PGES). The temporal and spatial properties of PGE_2 synthesis are determined by the regulated expression of each of the enzymes involved in this cascade. The first PGE synthase to be identified was microsomal PGE synthase 1 (mPGES1) [12], and to date it remains the only genetically-verified PGE synthase. Other putative PGE synthases including mPGES2 and cytosolic PGES (cPGES) have been described based on their capacity to generate PGE2 *in vitro* [13, 14]. However, recent evidence indicates that neither of these proteins function as PGE synthases *in vivo* [15, 16].

Studies using mPGES1$^{-/-}$ mice indicate that mPGES1 is responsible for the late phase of PGE_2 synthesis during inflammation via a pathway that depends on COX-2 [17]. Febrile responses to LPS are also mediated by mPGES1 [18] and mPGES1 is the source of PGE_2 that facilitates acute inflammatory pain [17]. Expression of mPGES1 is markedly upregulated in synovial tissues in arthritis [19], contributing to the pathogenesis of joint inflammation and swelling [17]. Taken together, these observations suggest that inhibition of mPGES1 might be useful for the treatment of inflammation and pain, providing a more limited therapeutic target than non-steroidal anti-inflammatory drugs (NSAIDs) or COX-2 inhibitors that cause a more global suppression of prostanoid synthesis. Moreover, since blockade of mPGES1 would not directly affect synthesis of PGI_2, an important vasodilator and anti-aggregant compound in the vasculature, adverse 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 [20]. On the other hand, studies of blood pressure regulation in mPGES1-deficient mice have yielded conflicting results, where in mPGES1-deficient mice have been reported to have normal [20-23] or reduced [24, 25] baseline blood pressures with normal or exaggerated responses to high salt feeding [21, 24]. Moreover, one group has reported that these animals have increased susceptibility to angiotensin II-dependent hypertension [24]. The genetic background of these various mPGES1-deficient mouse lines were all different. Since genetic background can have a marked influence on the phenotypes of genetically modified mice [26-28], 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 two 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.

MATERIALS AND METHODS

Animals

Production of inbred DBA/1 mice with targeted disruption of the mPGES1 (*Ptges1*) gene using embryonic stem (ES) cells derived from DBA/1lacJ inbred mice has been described previously [17]. 129/SvEv mPGES1$^{-/-}$ and $^{+/+}$ mice were generated by back-crossing the mPGES1 mutation onto the 129/SvEv genetic background for more than eight generations. Inbred DBA/1lacJ and 129/SvEv mPGES1$^{-/-}$ and $^{+/+}$ male mice generated by inter-crossing mPGES1$^{+/-}$ mice of each strain were used for the studies described here. Animals were maintained in the animal facility of the Durham Veterans Affairs Medical Center and studied between two and four months of age. The experimental procedures described below were approved by the respective IACUCs of the Durham VA and Duke University Medical Centers. Mice were fed a normal chow diet (0.4% NaCl; LabDiet, Richmond, IN).

Radiotelemetry measurements of intra-arterial pressure

Blood pressure was measured in conscious mice by radiotelemetry using TA11PA-C10 transmitters (Data Sciences International, St. Paul, MN) as described previously[29, 30]. Mice were allowed to recover for 7 days after surgery to regain their normal circadian rhythms before experiments were initiated. During blood pressure measurements, mice were housed in a monitoring room in the animal facility where quiet is maintained and no other activities are permitted. Data were collected continuously with sampling every 5 minutes for 10-second intervals using Dataquest A.R.T. software (Data Sciences International).

Angiotensin II-induced hypertension

Baseline blood pressure was recorded for three consecutive days, and then an osmotic minipump (Alzet model 2004, DURECT, Cupertino, CA) infusing Ang II (Sigma, St. Louis, MO) at a rate of 1,000 ng/kg/min was implanted subcutaneously, as described previously [30], and blood pressure measurements continued for three weeks.

Reverse transcription and real-time quantitative PCR

Total RNA was extracted (Tri-Reagent, Sigma) from mouse kidneys and reverse transcription and real-time PCR were performed as previously described [31]. Primers and dual-labeled probe (5'-FAM, 3'-TAMRA) targeting renin were synthesized based on previously published sequences[32]. Primer-probe sets for COX-1 (assay ID Mm00477214_m1) and COX-2 (assay ID Mm00478374_m1) were purchased from Applied Biosystems (Foster City, CA).

Urinary excretion of albumin and prostanoid metabolites

24-hour urine samples were collected by metabolic cage at baseline and after three weeks of Ang II infusion. Urine samples were centrifuged briefly to remove particulate matter, then immediately aliquoted and frozen at –80°C until assay. Urinary albumin excretion was measured using a specific ELISA assay for mouse albumin (Albuwell, Exocell, Philadelphia, PA). Stable prostanoid metabolites [13, 14-dihydro-15-keto-PGE_1/PGE_2 (prostaglandin E_2), TxB_2 (thromboxane A_2) , and 6-keto-$PGF_{1\alpha}$ (prostaglandin I_2)] in urine were measured using specific competitive enzyme immunoassays for each molecule (Cayman Chemical, Ann Arbor, MI). Creatinine levels in urine were assessed using an alkaline picrate assay (Creatinine Companion, Exocell, Inc., Philadelphia, PA).

Statistical analyses

All data are presented as mean ± SEM. Differences between groups were analyzed by unpaired t-test or two-way ANOVA followed by Bonferroni post-hoc test, as indicated, using GraphPad Prism software version 4.00 (GraphPad Software, San Diego, CA). Differences within groups,

before and after Ang II infusion, were analyzed by paired t-test. A p-value of less than 0.05 was considered significant.

RESULTS

We first measured blood pressures in the different mouse lines using radiotelemetry. At baseline, blood pressures in the 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 (MAP 105±1 vs. 107 ±1 mm Hg). By contrast, baseline blood pressures were significantly higher in the 129-mPGES1$^{-/-}$ animals (119±2 mm Hg) compared to the 129-WT controls (113±1 mm Hg; $p=0.008$).

It has been suggested that stimulation of PGE_2 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 30-40 mm Hg over the first 3-4 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 angiotensin 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 Figures 2A and B, the extent of the blood pressure elevation in the WT 129 mice was significantly greater than the WT DBA/1 animals (145±4 vs. 123±2 m 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 WT-129 littermates, (160±4 vs. 145±4 mm Hg, $p=0.01$; Fig. 2B).

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

To determine whether mPGES1 affects kidney damage induced by angiotensin II, we measured urinary albumin excretion at baseline and after three weeks of Ang II infusion. Basal levels of albumin excretion were similar in DBA and 129 WT mice (50±18 vs. 15±4 μg/24hr), and were unaffected by mPGES1 deletion in both strains of mice (DBA: 37±7; 129: 17±3 μg/24hr) (data not shown). With Ang II infusion, urinary albumin excretion increased only modestly in the WT DBA/1 animals (103±22 μg/24 hrs) and levels of albuminuria were basically unaffected by the absence of mPGES1 in the DBA/1-mPGES1$^{-/-}$ group (114±23 μg/24 hrs; Figure 4). By contrast, Ang II caused marked proteinuria in the WT 129 mice such that their levels of albumin excretion (514±106 μg/24 hrs) 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/24h; $p=0.003$ vs. WT).

To examine whether the absence of mPGES1 affected prostanoid metabolism and whether this might be affected by genetic background, we measured urinary prostanoid excretion in the various lines at baseline and during Ang II infusion. At baseline, urinary PGE_2 excretion was similar in DBA and 129 WT mice (813±120 vs. 808±137 pg/mg creatinine, Fig. 5). Mice lacking mPGES1 had reduced baseline PGE_2 excretion compared to WT, though 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; Fig. 5). Chronic infusion of Ang II caused significant increases in urinary excretion of PGE_2 metabolite in WT mice of both strains (DBA: 1968±498 pg/mg creatinine, p<0.01 vs. baseline; 129: 3962±1022 pg/mg creatinine, p<0.001 vs. baseline; Fig. 5). The levels of PGE_2 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 PGE_2 excretion during Ang II-dependent hypertension was attenuated in the mPGES1$^{-/-}$ animals of both strains compared to WT controls, though again the difference between 129-WT and mPGES1$^{-/-}$ animals was not statistically significant (DBA: 781±187 (mPGES1$^{-/-}$) vs. 1968±498 (WT) pg/mg creatinine, p=0.032; 129: 1508±585 (mPGES1$^{-/-}$) vs. 3962±1022 (WT) pg/mg creatinine, p=0.06).

We next measured urinary excretion of thromboxane (Tx) B_2, the stable metabolite of the potent vasocontrsictor prostanoid TxA_2 (Fig. 6, top panels), which has been implicated in the pathogenesis of Ang II-dependent hypertension [34-36]. At baseline, there were no differences in excretion of urinary TxB_2 between WT DBA/1 and 129 mice (1542±391 vs. 1976±529 pg/mg creatinine). On the DBA/1 background, absence of mPGES1 has no effect on TxB_2 excretion at baseline (1376±232 vs.1542±391 pg/mg creatinine, p=NS), whereas urinary TxB_2 was higher in 129-mPGES1$^{-/-}$ mice compared to the 129 WT controls, though the difference was not statistically significant (3402±796 vs. 1976±529: p=0.074). As shown in Figure 6 (top panels), chronic infusion of Ang II had no effect on urinary TxB_2 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 TxB_2 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) (Fig. 5, right panel).

We measured urinary excretion of 6-keto-$PGF_{1\alpha}$, the stable metabolite of prostacyclin, a potent vasodilator and anti-thrombotic prostanoid (Fig. 6). At baseline, excretion of urinary 6-keto-$PGF_{1\alpha}$ was very similar in all 4 experimental groups and there was no difference in excretion of the PGI_2 metabolite between WT and mPGES1-deficient mice on the DBA/1 (5350±1264 vs.6948±924 pg/mg creatinine, p=NS) or 129 backgrounds (5223±1291 vs. 5218±1153, p=NS). As shown in Figure 6, urinary 6-keto-$PGF_{1\alpha}$ excretion in the DBA lines was virtually unaffected by chronic infusion of angiotensin 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-$PGF_{1\alpha}$ 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$^{-/-}$ mice (5218±1153 to 35517±5463 pg/mg creatinine, p<0.001).

We next measured mRNA levels for the cyclo-oxygenase enzymes COX-1 and -2 (Fig. 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 vs. 130±17% for DBA/1 and 100 ±9 vs. 73±5% of WT for 129). Infusion of angiotensin II did not affect COX-1 mRNA abundance in the DBA/1 mice (WT 111±26 vs. mPGES1−/− 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$^{-/-}$ animals (146±21% of WT baseline, p<0.05), although this increase was statistically significant only in the mPGES1$^{-/-}$ group. As shown in Figure 7, baseline COX-2 expression was 2-fold higher in kidneys of DBA/1 mPGES1$^{-/-}$ animals than their WT controls (100±18 vs. 204±38% of WT; p<0.05), but there was no difference in COX-2 mRNA levels in the 129 mPGES1$^{-/-}$ mice at baseline (100±29 vs. 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 vs. mPGES1−/− 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$^{-/-}$ animals (279±36% of WT baseline, p<0.05), but this increase was statistically significant only in the 129-mPGES1$^{-/-}$ group.

DISCUSSION

A role for PGE_2 in blood pressure homeostasis was first suggested by studies documenting its vasodilator actions in the systemic circulation [37, 38]. Infusion of PGE_2 into the kidney commonly causes renal vasodilation [39, 40]. 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 by the kidney are renal medullary interstitial cells and collecting duct [8, 41, 42]. 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 utilized. 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 [21]. These findings were similar to the report from the FitzGerald group demonstrating normal blood pressures in mice lacking mPGES1 [22]. By contrast, Yang and associates found that mPGES1-deficient mice developed hypertension with high salt feeding [24]. Moreover, they also reported that mPGES1-deficient mice were more susceptible to hypertension caused by chronic infusion of angiotensin II, manifesting exaggerated oxidative stress in this setting [25]. On the other hand, subsequent work by FitzGerald and associates showed that deletion of mPGES1 in LDL receptor-deficient mice does not affect their hypertensive response to chronic infusions of angiotensin II but protects them from aortic aneurysm formation and significantly reduces oxidative stress [23]. 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 two 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 [17, 26-28]. We found that wild-type 129/SvEv mice have higher baseline blood pressure than wild-type 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 wild-type 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 angiotensin II infusion that were virtually identical to the DBA/1 wild-type group, the severity of hypertension was substantially augmented in the 129-mPGES1$^{-/-}$ mice compared to the 129 wild-type controls (Figure 2). Our findings are generally consistent with previous studies indicating that there are strain-specific genetic modifiers enhancing susceptibility to hypertension in 129 mice [28, 43, 44]. Moreover, our studies indicate a significant impact of mPGES1 to attenuate hypertension in the 129 but not the DBA/1 strain. This may explain some of the inconsistencies in blood pressure phenotypes previously reported for mPGES1-deficient mouse lines, which consisted of varying contributions from DBA/1 and 129 backgrounds [20-25].

Enhanced hypertension in the 129 animals was also accompanied by exaggerated kidney injury as reflected by increased albumin excretion during angiotensin II infusion. This was apparent in the 129 wild-type mice compared to the wild-type DBA/1 group, consistent with enhanced susceptibility to kidney injury that have been observed in this strain [26-28]. Furthermore, albuminuria induced by Ang II infusion was dramatically enhanced in the mPGES1-deficient 129 mice. Since the extent of blood pressure elevation was also more severe in the 129-mPGES1$^{-/-}$, it is not clear whether the worsening proteinuria was due to more severe hypertension or reflected a vulnerability to kidney damage due to the absence of mPGES1. By contrast, DBA/1 wild-type mice developed only modest albuminuria during angiotensin 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.

PGE_2 synthesis is stimulated during hemodynamic stress, and it has been suggested that increased levels of PGE_2 may be a compensatory response to attenuate the development of hypertension[45]. Chronic infusion of angiotensin II stimulated increased urinary excretion of PGE metabolite in wild-type mice of both DBA/1 and 129 strains. Elimination of mPGES1 significantly attenuated PGE_2 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 PGE_2 production in response to angiotensin II. However, there is significant residual production of PGE_2 in both lines of mPGES1$^{-/-}$ mice suggesting that there are robust pathways for PGE_2 synthesis in vivo that do not require mPGES1. Despite a similar diminution of PGE_2 excretion and higher absolute levels of urinary PGE_2 metabolite with angiotensin II infusion in mPGES1$^{-/-}$ 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 [34-36], might explain the variable blood pressure responses that we observed. At baseline, urinary excretion of TxB_2, the stable metabolite of TxA_2, were similar in all of the DBA/1 and 129 lines. Angiotensin 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-mPGES1-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 angiotensin 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-mPGES1$^{-/-}$ mice is not completely clear from our studies. While this cannot be explained by altered renin responses, differences in COX expression and prostanoid production in response to angiotensin 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 mPGES1$^{-/-}$ mice since previous studies by our group using thromboxane receptor (TP)-deficient mouse lines have shown that enhanced generation of TxA_2 contributes to blood pressure elevation and end-organ damage in angiotensin II-dependent hypertension [34]. However, the physiological consequences of altered prostanoid 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 nine-fold and TxB_2/6-keto-$PGF_{1\alpha}$ is increased 2-fold during angiotensin II infusion in the 129-*mPges1*$^{-/-}$ mice compared to the 129 wild-type controls. This indicates a tendency toward enhanced thromboxane generation in the mPGES1-deficient mice on the 129 strain mice that could contribute to their susceptibility to develop hypertension.

Expression of mPGES1 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 (EP) receptor isoforms suggest that PGE_2 is responsible for inflammatory pain [46] and fever [47]. It is perhaps not surprising that genetic deletion of mPGES1 recapitulates many of the beneficial anti-inflammatory actions of NSAIDs. Accordingly, it has been suggested that small molecule inhibitors of mPGES1 might be useful anti-inflammatory 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 mPGES1 blockade on blood pressure may be influenced by genetic background and associated stimulation of TxA_2 production.

PERSPECTIVES

mPGES1 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 mPGES1 may produce variable cardiovascular effects depending on genetic and environmental factors. Identification of genetic variables that influence the renal and cardiovascular response to mPGES1 inhibition may facilitate safe and effective therapies targeting this enzyme.

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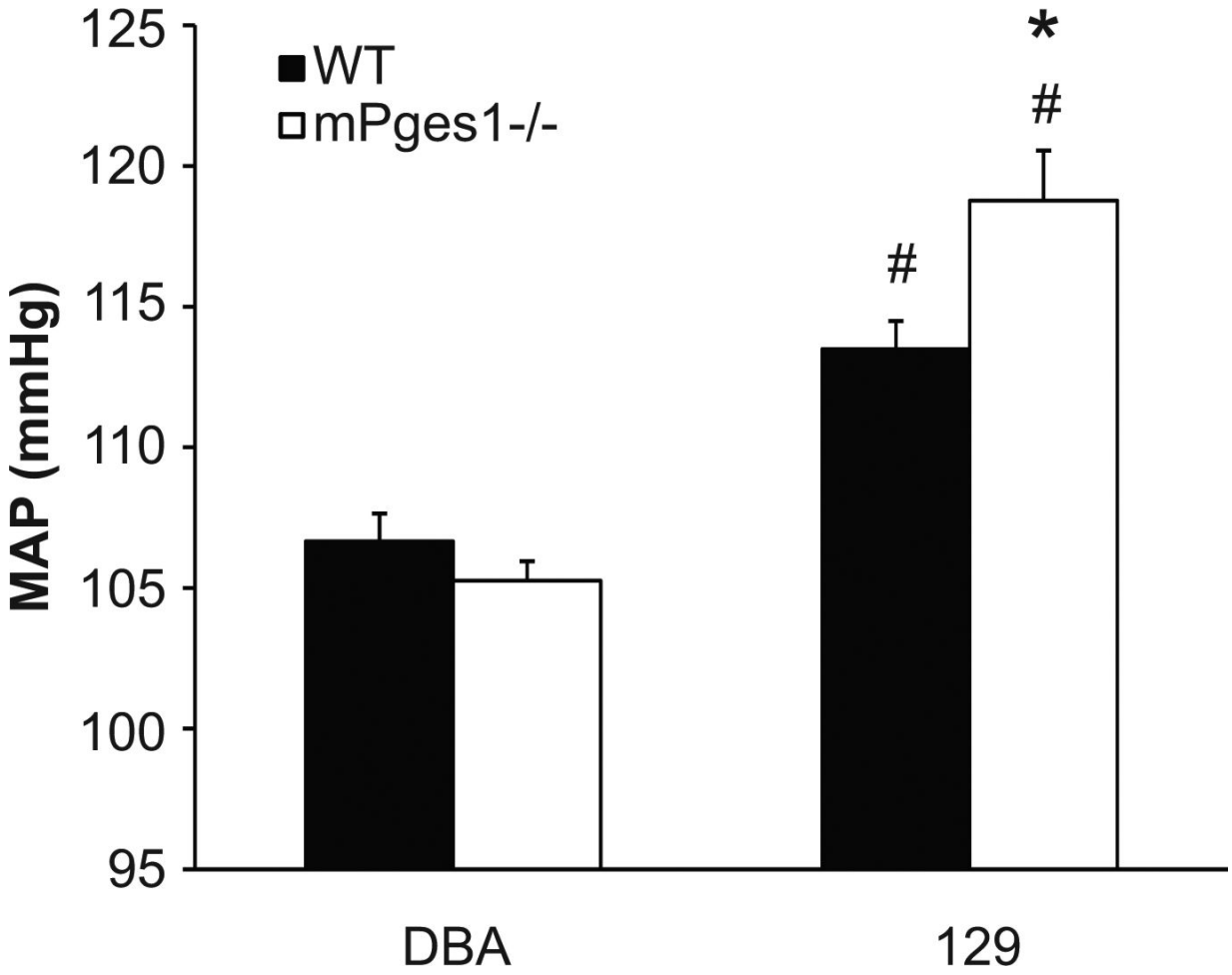

Figure 1.

Baseline mean arterial pressure (MAP) in wild-type (black bars, n=8-10) and mPGES1$^{-/-}$ (white bars, n=5-9) mice on the DBA/1lacJ and 129/SvEv genetic backgrounds. Blood pressure was measured continuously in conscious animals by radiotelemetry. Data are 24-hour mean values for three consecutive days of recordings. *$p<0.05$ vs. WT, $^{\#}$ $p<0.05$ vs. DBA/1lacJ by two-way ANOVA with Bonferroni post test.

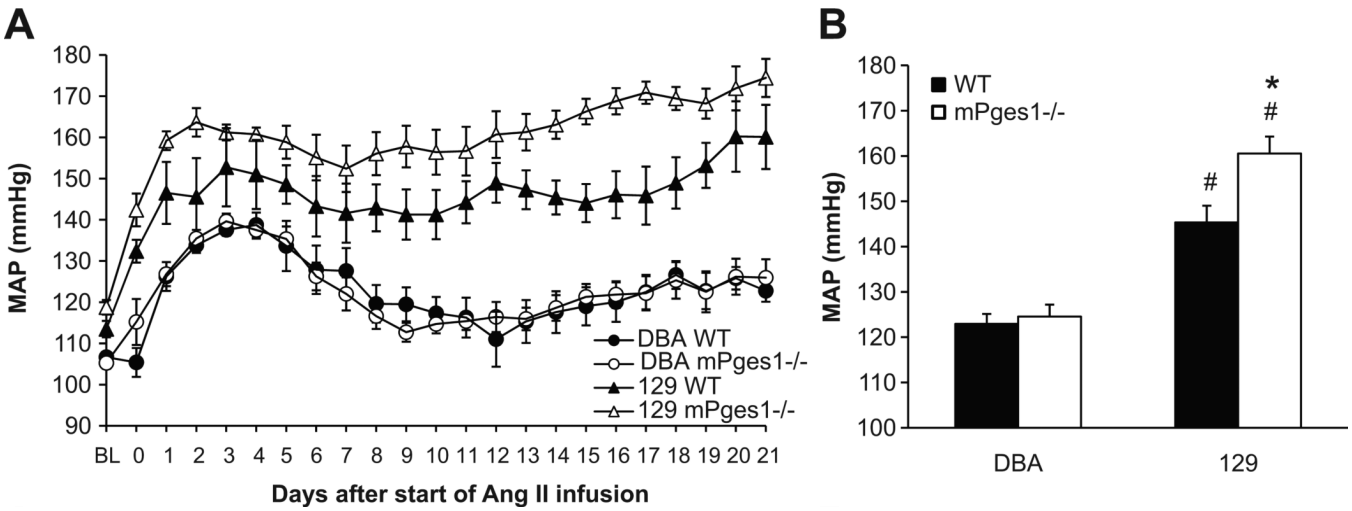

Figure 2.

Angiotensin II-dependent hypertension in DBA/1lacJ and 129/SvEv mice in the presence and absence of mPGES1. Ang II (1000 ng/kg/min) was infused for three weeks by osmotic mini-pump 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 three weeks of Ang II infusion in DBA/1 (WT, n=9; 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 two-way ANOVA with Bonferroni post test.

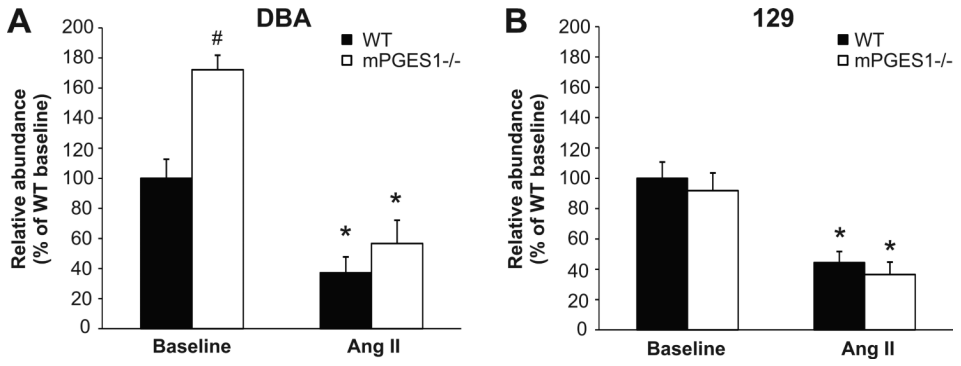

Figure 3.

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 three weeks of Ang II infusion. Data are expressed as % of DBA/1 and 129 WT baseline. * $p<0.05$ vs. baseline, $^{\#}$ $p<0.05$ vs. WT by two-way ANOVA with Bonferroni post test.

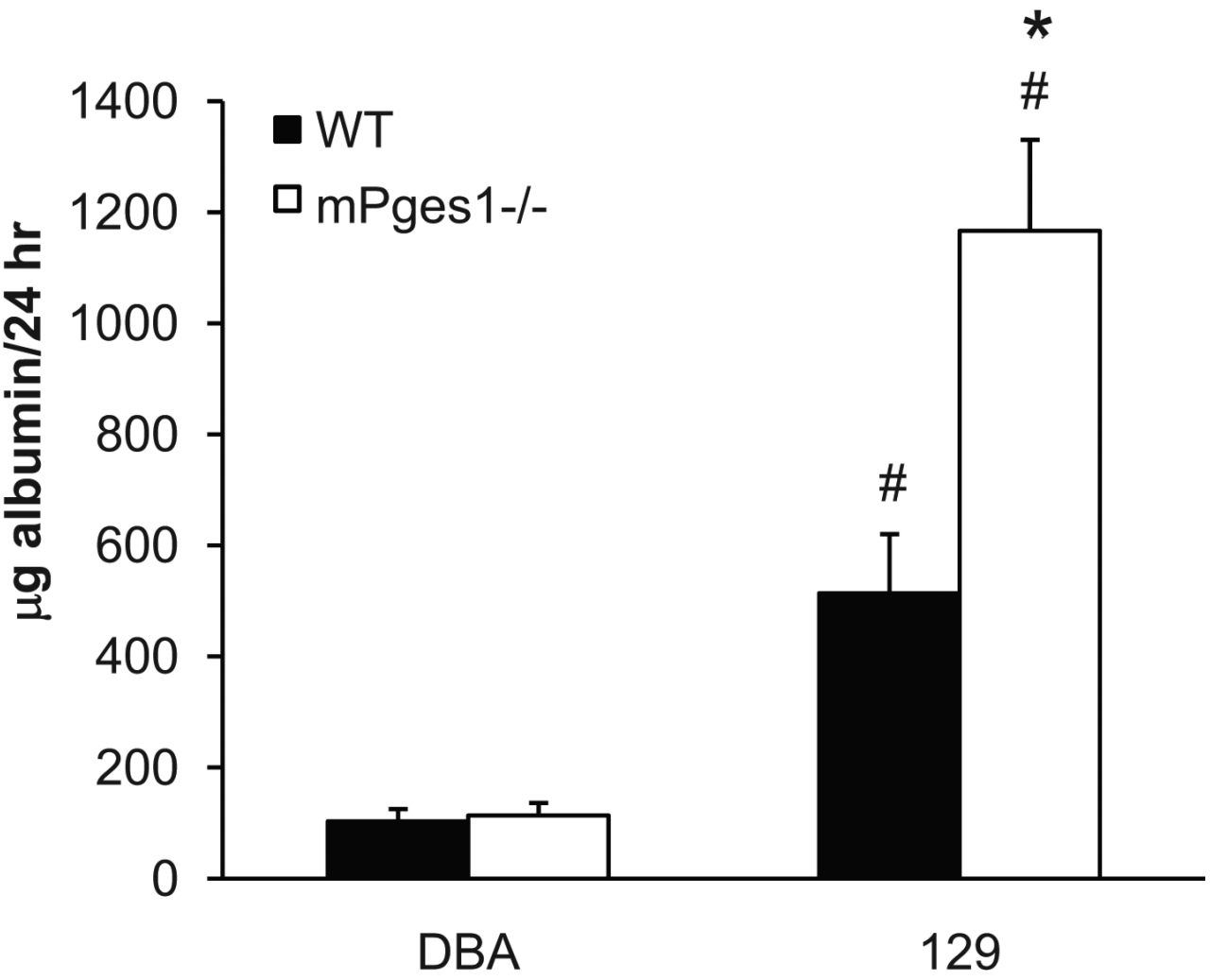

Figure 4.
Urinary albumin excretion after chronic Ang II infusion in DBA/1 and 129/SvEv WT and mPGES1−/− animals. Albumin levels were measured by ELISA in 24-hour urine samples after three weeks of Ang II infusion. * $p<0.05$ vs. WT, # $p<0.05$ vs. DBA/1 by two-way ANOVA with Bonferroni post test.

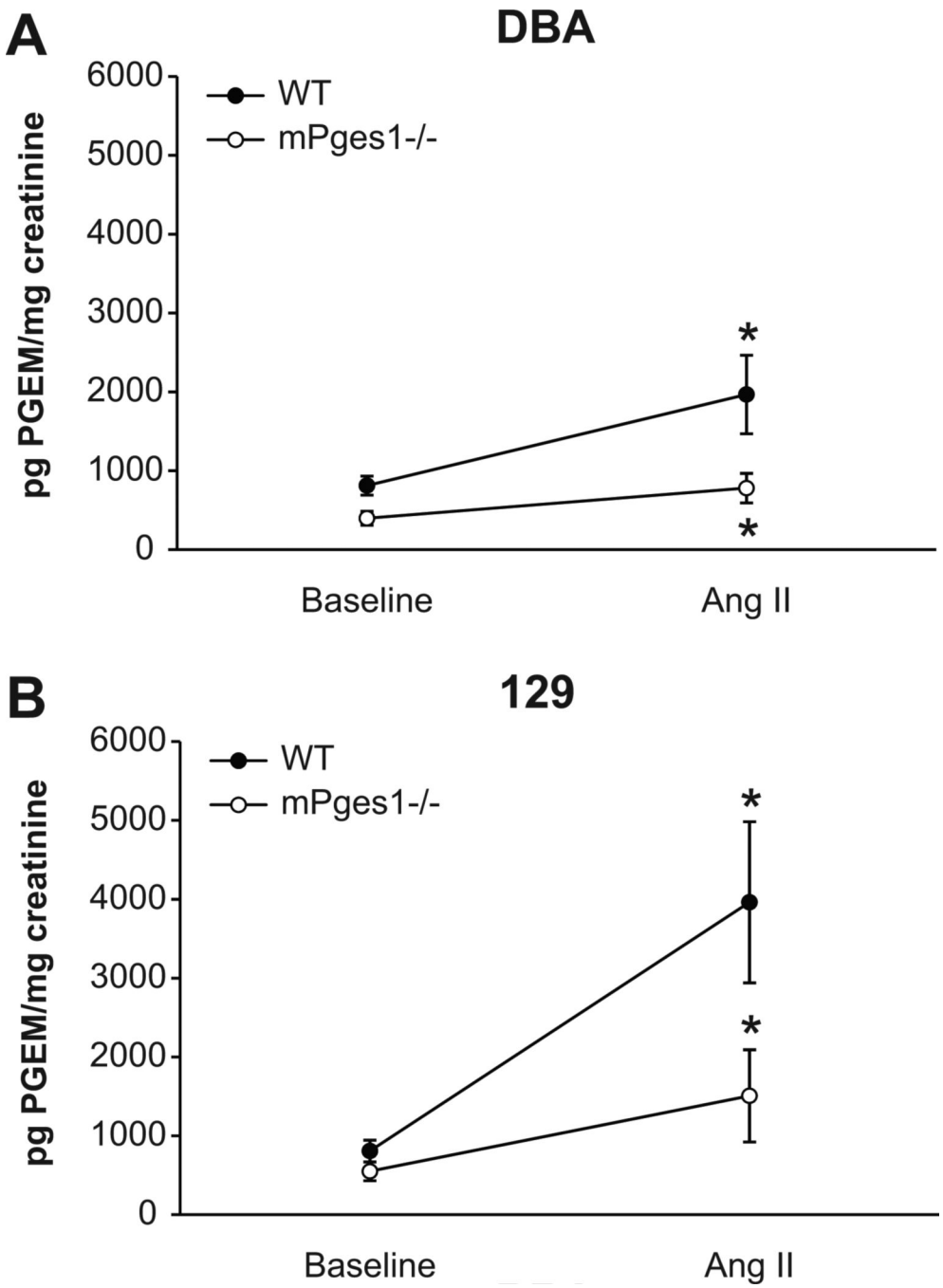

Figure 5.

Urinary PGE metabolite excretion. PGE metabolite levels were measured in 24-hour urine samples before and after three weeks of Ang II infusion in DBA/1lacJ (left panel) and 129/SvEv (right panel) mice comparing WT (black circles) and mPGES1-deficient (white circles) mice. * $p<0.05$ vs. baseline by paired t-test.

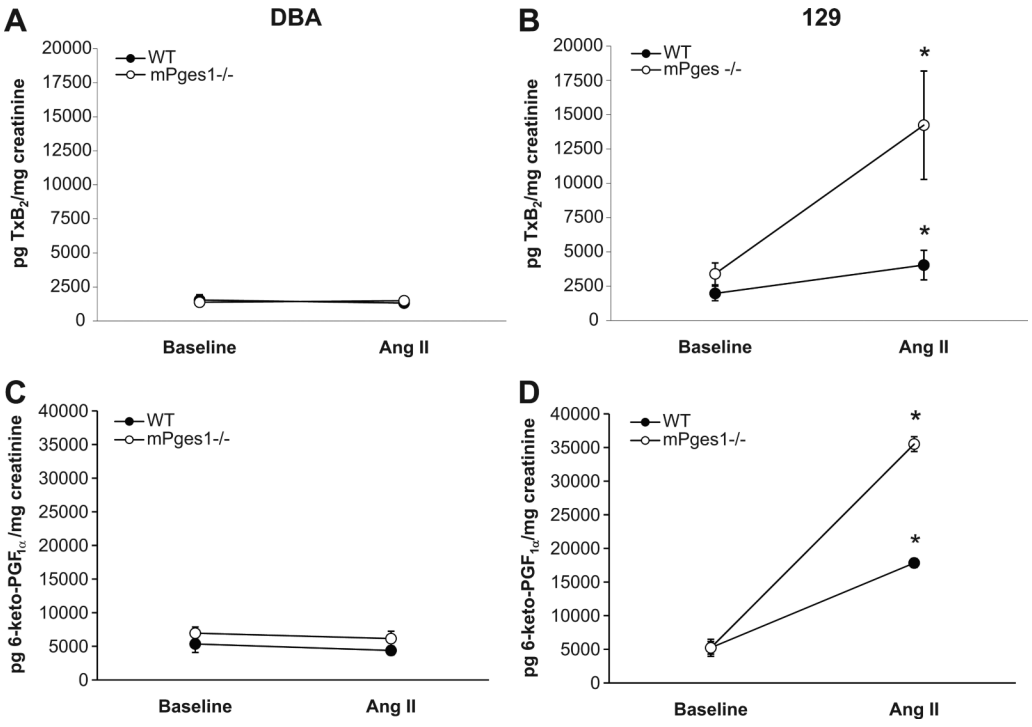

Figure 6.

Urinary excretion of thromboxane A_2 and prostacylin metabolites. Levels of TxB_2 (top panels) and 6-keto-$PGF_{1\alpha}$ (bottom panels) were measured in 24-hour urine samples by enzyme immunoassay in DBA/1 and 129 mice. * $p<0.05$ vs. baseline by paired t-test.

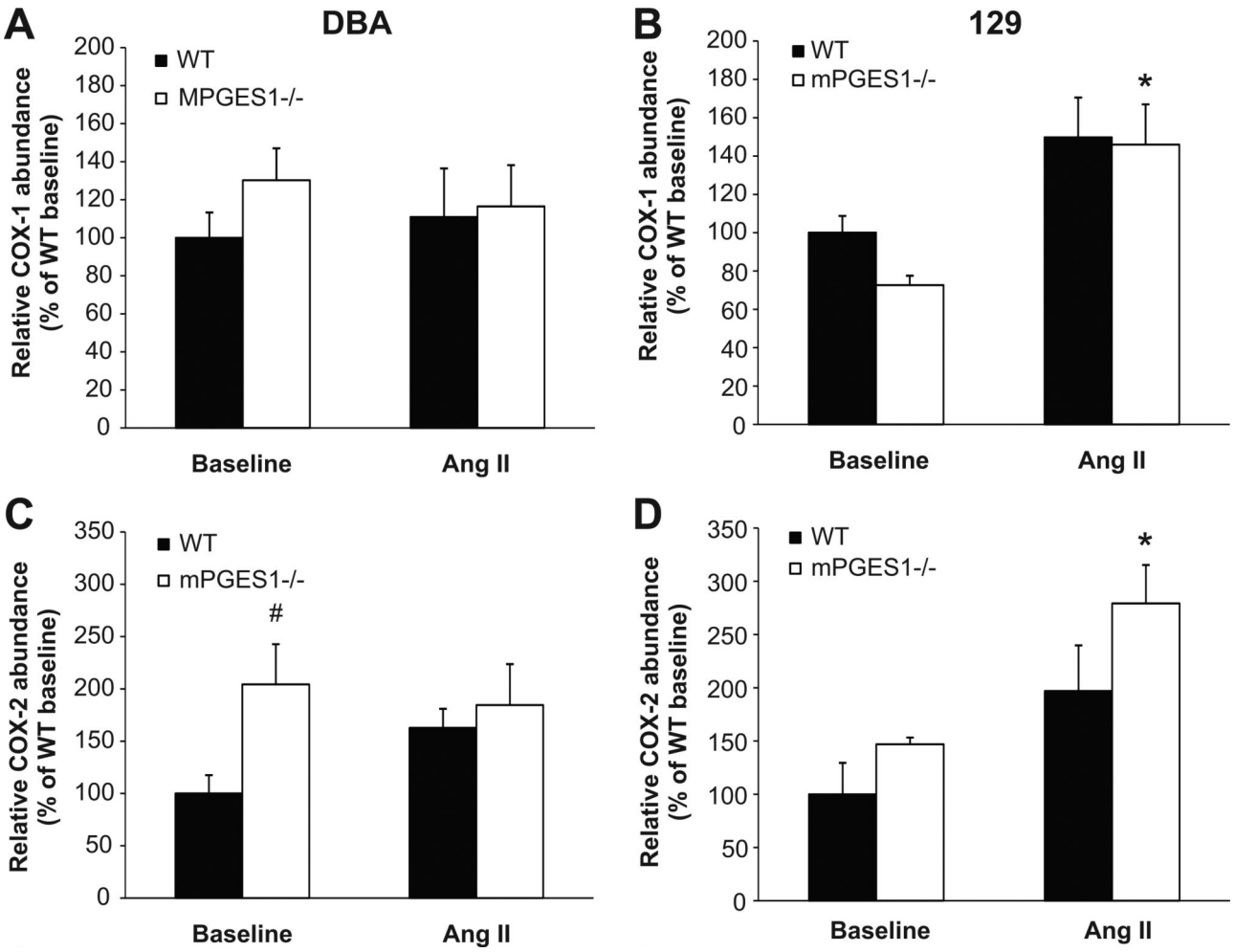

Figure 7.

Expression of cyclo-oxygenase (COX) isoforms COX-1 and COX-2 in mouse kidneys. Total RNA was isolated from kidneys and levels of mRNA for COX-1 (upper panels) and COX-2 (lower panels) were measured by real-time RT-PCR at baseline and after three weeks of Ang II infusion in DBA/1 (A,C) and 129/SvEv (B,D) mice. * $p<0.05$ vs. baseline by two-way ANOVA with Bonferroni post test; # $p<0.05$ vs. WT by two-way ANOVA with Bonferroni post test.