Contribution of prostaglandin EP<sub>2</sub> receptors to renal microvascular reactivity in mice

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Imig, John D., Matthew D. Breyer, and Richard M. Breyer. Contribution of prostaglandin EP<sub>2</sub> receptors to renal microvascular reactivity in mice. Am J Physiol Renal Physiol 283: F415–F422, 2002.—The present studies were performed to determine the contribution of EP<sub>2</sub> receptors to renal hemodynamics by examining afferent arteriolar responses to PGE<sub>2</sub>, butaprost, sulprostone, and endothelin-1 in EP<sub>2</sub> receptor-deficient male mice (EP<sub>2</sub>−/−). Afferent arteriolar diameters averaged 17.8 ± 0.8 μm in wild-type (EP<sub>2</sub>+/+) mice and 16.7 ± 0.7 μm in EP<sub>2</sub>−/− mice at a renal perfusion pressure of 100 mmHg. Vessels from both groups of mice responded to norepinephrine (0.5 μM) with similar 17–19% decreases in diameter. Diameters of norepinephrine-preconstricted afferent arterioles increased by 7 ± 2 and 20 ± 6% in EP<sub>2</sub>+/+ mice in response to 1 μM PGE<sub>2</sub> and 1 μM butaprost, respectively. In contrast, afferent arteriolar diameter of EP<sub>2</sub>−/− mice decreased by 13 ± 3 and 16 ± 6% in response to PGE<sub>2</sub> and butaprost. The afferent arteriolar vasoconstriction to butaprost in EP<sub>2</sub>−/− mice was eliminated by angiotensin-converting enzyme inhibition. Sulprostone, an EP<sub>1</sub> and EP<sub>3</sub> receptor ligand, decreased afferent arteriolar diameter in both groups; however, the vasoconstriction in the EP<sub>2</sub>−/− mice was greater than in the EP<sub>2</sub>+/+ mice. Endothelin-1-mediated afferent arteriolar diameter responses were enhanced in EP<sub>2</sub>−/− mice. Afferent arteriolar diameter decreased by 29 ± 7% in EP<sub>2</sub>−/− and 12 ± 7% in EP<sub>2</sub>+/+ mice after administration of 1 nM endothelin-1. These results demonstrate that the EP<sub>2</sub> receptor mediates a portion of the PGE<sub>2</sub> afferent arteriolar vasodilation and buffers the renal vasoconstrictor responses elicited by EP<sub>1</sub> and EP<sub>3</sub> receptor activation as well as endothelin-1.

THE REGULATION OF WATER and electrolyte homeostasis is dependent on the renal hemodynamic and tubular transport actions of PGE<sub>2</sub> (5, 14, 25, 42). PGE<sub>2</sub> is the major renal cyclooxygenase (COX)-derived metabolite in the kidney and the PGE<sub>2</sub> receptors (EP) are abundantly expressed in the kidney (5, 7, 8). Four seven-transmembrane-spanning domain, G protein-coupled EP receptors have been identified (5, 7, 8). The intracellular signaling mechanisms for the EP receptors have been characterized and activate mechanisms that would either relax or contract smooth muscle (5, 14). Overall, PGE<sub>2</sub> has been demonstrated to increase renal blood flow and glomerular filtration rate but the contribution of EP receptors to the control of renal hemodynamics remains unresolved.

An important role for the EP<sub>2</sub> receptors in regulating fluid and electrolyte homeostasis has been suggested by studies in mice with targeted disruption of these receptors (21, 38, 43). Disruption of the EP<sub>2</sub> receptor in mice does not alter renal blood flow but does unmask a systemic vasoconstriction in response to PGE<sub>2</sub> (3, 43). These mice lacking EP<sub>2</sub> receptors develop salt-sensitive hypertension (20). Thus further investigation of the renal microvascular actions of PGE<sub>2</sub> is of extreme interest in these mice. The purpose of the present study was to determine the contribution of EP<sub>2</sub> receptors to renal hemodynamics by examining afferent arteriolar responses to PGE<sub>2</sub>, selective EP receptor agonists, and endothelin-1 in mice lacking EP<sub>2</sub> receptors.

MATERIALS AND METHODS

Chemical reagents. Sulprostone, PGE<sub>2</sub>, and butaprost were purchased from Cayman Chemical. Norepinephrine (Levolphed) was obtained from Winthrop Pharmaceuticals. Endothelin-1 was purchased from Phoenix Pharmaceuticals. Enalaprilat was a gift from Merck Sharp and Dohme. Indomethacin and all other reagents were purchased from Sigma.

Animal preparation. EP<sub>2</sub> receptor-deficient mice were generated at Vanderbilt University as previously described (21). F<sub>2</sub> wild-type (EP<sub>2</sub>+/+) and EP<sub>2</sub>-null (EP<sub>2</sub>−/−) mice were littermates produced from intercrossing F<sub>1</sub> heterozygous (EP<sub>2</sub>+/−) mice. All mice were weaned at 3 wk of age and fed a standard chow diet. Genotypes of the mice were routinely determined by Southern analysis of genomic tail DNA. The wild-type (4.3 kb) and recombinant (7.5 kb) XbaI fragments were identified by using a 3′ XbaI/SacI fragment as a probe. Animals were housed for at least 2 wk at the Tulane University School of Medicine vivarium. The Vanderbilt University and Tulane Advisory Committee for Animal Resources approved all experi-

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Vascular preparation. Experiments were performed in male EP2+/+ and EP2−/− mice weighing an average of 33 ± 1 and 32 ± 1 g, respectively. Mice were anesthetized with a combination of thiothubarbital (Inactin; 100 mg/kg ip) and ketamine (Ketaset; 10 mg/kg ip), and a midline abdominal incision was made. The right renal artery was cannulated via the superior mesenteric artery, and the kidney was immediately perfused with Tyrode solution containing 6% albumin and a mixture of L-amino acids (15). All protocols were conducted in the juxtaglomerular microvascular preparation perfused with the cell-free Tyrode solution containing 6% albumin. We previously demonstrated that the main difference between a cell-free and red blood cell-containing solution is that nitric oxide levels are elevated in a cell-free perfusate (16). The Tyrode solution was stirred continuously in a closed reservoir that was pressurized with a 95% O2-5% CO2 tank. The kidney was removed from the mouse and maintained in an organ chamber at room temperature throughout the isolation and dissection procedure. The juxtaglomerular microvasculature was isolated for study as previously described (15). The organ chamber was then warmed, and the tissue surface was continuously superfused with Tyrode solution containing 1% albumin at 37°C. Renal artery perfusion pressure, measured at the tip of the cannula, was set to 100 mmHg.

Determination of afferent arteriolar diameter was accomplished using transillumination videomicroscopy as previously described (15). The tissue was transilluminated, and the focused image was converted to a video signal by a high-resolution Newvicon camera. This video signal was electronically enhanced and recorded on videotape for later analysis. Afferent arteriolar inside diameters were measured at 15-s intervals using a digital image-shearing monitor. The image-shearing device is accurate to within 0.2% of the screen width or 0.2 μm, and measurement reproducibility is within 0.5 μm. The average diameter during the final 2 min of each 5-min treatment period was used for statistical analysis of steady-state responses.

Afferent arteriolar diameter response to PGE2. After a 20-min equilibration period, baseline diameter measurements of the afferent arteriole were made. Norepinephrine (0.5 μM) was added to the perfusate to elevate basal vascular tone. The endogenous ligand PGE2 (1 μM) was added to the perfusate, and vessel diameter changes were monitored for 5 min. In additional experiments, the influence of the renin-angiotensin system on the afferent arteriolar diameter response was evaluated. For these studies, the angiotensin-converting enzyme inhibitor enalaprilat (1 mg ip) was administered to the mice (17). One hour after the injection, the kidney was harvested and the afferent arteriolar response to PGE2 was determined as described above.

Afferent arteriolar response to the EP receptor activation with butaprost or sulprostone. After a 20-min equilibration period and baseline diameter measurements, the afferent arteriole was preconstricted with norepinephrine (0.5 μM). The arteriole was subsequently exposed to increasing concentrations of an EP2 receptor-selective ligand, butaprost (0.01–1 μM) (7, 8, 14, 25), and diameter change was monitored for 5 min at each concentration. In a separate series, the afferent arteriolar diameter response to butaprost was determined in enalaprilat-treated EP2+/+ and EP2−/− mice.

The afferent arteriole diameter response to an EP1 and EP3 receptor ligand, sulprostone (7, 8, 14, 25), was determined in EP2−/− mice. Administration of norepinephrine (0.5 μM) to the perfusate resulted in an elevated vascular tone. Sulprostone (0.01–1 μM) was superfused, and the afferent arteriolar diameter changes were monitored.

Involvement of the EP2 receptor in the afferent arteriolar vasodilator response to endothelin-1. After a 20-min equilibration period, baseline diameter measurements of the afferent arteriole were made. Endothelin-1 (0.1–10 nM) was then administered in increasing concentrations, and diameter changes were monitored. In a separate series, the concentration-response profile to endothelin-1 was determined in the presence of the nonselective COX inhibitor indomethacin (10 μM) (15). Indomethacin was added to the perfusate and superfusate for 20 min to ensure complete tissue blockade (15).

Statistical analysis. In all experiments, steady-state diameter was attained by the end of the second minute, and the average diameter of the third to fifth minute of each treatment period was used for graphical representation. Data are presented as means ± SE. The basic design for each treatment protocol is a prospective randomized controlled trial with repeated measures over time for the independent groups. Standard parametric change-from-baseline analyses within each group were conducted for each of the outcome measures. Change scores were computed and used in between-group hypothesis testing (ANOVA). Post hoc multiple comparisons were made using standard statistical Student-Newman-Keuls methods to adjust the “comparison-wise” error rate. A value of P < 0.05 was considered statistically significant.

RESULTS

Afferent arteriolar diameter response to PGE2 in EP2+/+ and EP2−/− mice. Consistent with previous reports, body weight was similar between the groups and averaged 33 ± 1 g in EP2+/+ and 32 ± 1 g in EP2−/− mice. Afferent arteriolar diameter at a renal perfusion pressure of 100 mmHg was unaltered by the absence of EP2 receptors. Diameter of the afferent arteriole averaged 16.7 ± 0.7 μm (n = 39) in EP2−/− compared with 17.8 ± 0.8 μm (n = 40) in EP2+/+ mice. Norepinephrine decreased preglomerular diameter to the same extent in EP2+/+ and EP2−/− mice. Afferent arteriolar diameter decreased by 17 ± 3% (n = 29) in EP2+/+ and 18 ± 4% (n = 27) in EP2−/− mice in response to perfusion of 0.5 μM norepinephrine.

In the first series of experiments, the response of the afferent arteriole to the endogenous EP2 receptor ligand PGE2 was determined. Afferent arteriolar diameter decreased from 17.6 ± 0.6 to 14.6 ± 0.8 μm (n = 12) in EP2+/+ and from 16.5 ± 0.8 to 13.4 ± 0.7 μm (n = 11) in EP2−/− mice after norepinephrine administration. In EP2+/+ mice, diameter of the afferent arteriole increased in response to superfusion of 1 μM PGE2 in five of the six vessels studied. In contrast, 1 μM PGE2 decreased preglomerular vessel caliber by 13 ± 4% (n = 5) in mice lacking EP2 receptors (Fig. 1). The afferent arteriolar response to PGE2 was determined in additional experiments to evaluate the involvement of the renin-angiotensin system. Enalaprilat treatment did not alter baseline values, and afferent arteriolar diameter averaged 18.4 ± 1.9 (n = 6) and 17.4 ± 1.2 μm (n = 6) in EP2+/+ and EP2−/− mice, respectively. PGE2 increased afferent arteriolar diameter in six of the six vessels studied, and the
Interestingly, the afferent arteriolar vasoconstrictor response to PGE2 reversed to a 6 ± 3% increase in vessel diameter after angiotensin-converting enzyme inhibition in mice lacking EP2 receptors (Fig. 2). PGE2 significantly increased diameter from control in four afferent arterioles, and diameter did not change in the other two afferent arterioles taken from enalaprilat-treated EP2−/− mice.

**Afferent arteriolar diameter response to butaprost in EP2+/+ and EP2−/− mice.** Figure 3 depicts the preglomerular vascular response to the selective EP2 receptor ligand butaprost in EP2+/+ and EP2−/− mice. The diameter of norepinephrine-precontracted afferent arterioles increased by 20 ± 6% (n = 6) in response to 1 µM butaprost in EP2+/+ mice. Similar to the response to PGE2, superfusion of 1 µM butaprost constricted the preglomerular vessel caliber by 16 ± 6% (n = 6) in mice lacking EP2 receptors. Additional experiments were performed to determine the involvement of the renin-angiotensin system to the butaprost-mediated afferent arteriolar vasoconstriction in EP2−/− mice. After enalaprilat treatment, afferent arteriolar diameters were not different from untreated mice and averaged 18 ± 2 µm (n = 5) in EP2+/+ and 17 ± 1 µm (n = 5) in EP2−/− mice. Angiotensin-converting enzyme inhibition eliminated the preglomerular vasoconstrictor response in EP2−/− mice but did not significantly alter the vasodilatory response to butaprost in EP2+/+ mice (Fig. 4).

**Afferent arteriolar diameter response to sulprostone in EP2+/+ and EP2−/− mice.** The preglomerular vascular response to EP1 and EP3 receptor activation with sulprostone is depicted in Fig. 5. The afferent arteriolar diameter response to sulprostone was significantly greater in EP2−/− mice compared with that of EP2+/+ mice. Sulprostone (1 µM) decreased afferent arteriolar diameter by 7 ± 2% (n = 6) in EP2+/+ mice and by 17 ± 3% (n = 5) in mice lacking EP2 receptors. Enhanced afferent arteriolar reactivity to endothelin-1 in EP2−/− mice. Figure 6 depicts the afferent arteriolar vasoconstrictor response to endothelin-1 in EP2+/+ and EP2−/− mice. Afferent arteriolar diameters decreased after superfusion of endothelin-1 and reached a steady-state diameter by the end of the second minute. The preglomerular vascular response to endothelin-1 was significantly enhanced in mice lacking EP2 receptors. Afferent arteriolar diameter decreased by 12 ± 7% (n = 6) in EP2+/+ and 29 ± 7% (n = 5) in EP2−/− mice after administration of 1 nM endothelin-1.

The effects of the COX inhibition on endothelin-1 afferent arteriolar vasoconstriction were evaluated to
determine whether the generation of endogenous COX metabolites was responsible for the difference between EP2+/+ and EP2−/− mice. In the presence of indomethacin, afferent arteriolar diameter averaged 18.3 ± 1.9 μm (n = 5) in EP2+/+ and 17.0 ± 0.9 μm (n = 6) in EP2−/− mice. Afferent arteriolar diameter in EP2+/+ mice decreased by 4 ± 1, 11 ± 3, and 24 ± 4% in response to 0.1, 1, and 10 nM endothelin-1. The preglomerular vascular response to endothelin-1 during COX inhibition was attenuated in EP2+/+ mice and became similar to that observed in EP2+/− mice. In mice lacking EP2 receptors, afferent arteriolar diameter decreased by 7 ± 4, 13 ± 4, and 21 ± 3% in response to 0.1, 1, and 10 nM endothelin-1 (Fig. 6).

**DISCUSSION**

Studies in mice lacking EP2 receptors point to a critical role for these receptors in the maintenance of renal blood flow and water homeostasis (21, 38, 43). The present study focused on the contribution of the EP2 receptor to the control of renal microvascular function. We found that PGE2 or the EP2 receptor ligand butaprost when administered to wild-type mice resulted in an increase in afferent arteriolar diameter. In contrast, PGE2 and butaprost decreased afferent arteriolar vessel caliber in EP2−/− mice. The vasoconstriction in response to PGE2 and butaprost in mice lacking EP2 receptors was eliminated by angiotensin-converting enzyme inhibition. These findings suggest that PGE2-mediated stimulation of the renin-angiotensin system in EP2−/− mice was responsible for the afferent arteriolar vasoconstriction observed in these mice. In addition to the afferent arteriolar vasoconstrictor response to the EP1 and EP3 receptor agonists, sulprostone was enhanced in mice lacking EP2 receptors. Endothelin-1 also resulted in a greater decrease in preglomerular diameter in EP2−/− mice. The enhanced vasoconstrictor response to endothelin-1 in mice lacking EP2 receptors appears to be COX-dependent because indomethacin eliminated this difference between EP2+/+ and EP2−/− mice. Overall, the results of these studies suggest that EP2 receptors help sustain renal blood flow.

The biological actions of PGE2 are mediated via activation of one of four EP receptors (5). EP receptors are abundant throughout the kidney and are expressed in the renal microcirculation (5, 31, 37, 43). Molecular and pharmacological characterization of four different EP receptors, designated EP1−4, have been completed (5, 39). Activation of vascular EP1 and EP3 receptors would be expected to contract smooth muscle cells. EP1 receptors act via the inositol trisphosphate (IP3), diacylglycerol (DAG), and protein kinase C (PKC) pathway.
and EP3 receptors decrease cAMP and increase rho (1, 2, 24). Activation of either EP2 or EP4 receptors results in an increase in rabbit and rat preglomerular vessel cAMP levels and results in relaxation of vascular smooth muscle (5, 10, 17, 33). In many studies, PGE2 has been demonstrated to increase renal blood flow (5, 14, 25); however, under certain experimental conditions renal vasoconstriction has been observed during administration of PGE2 (19). These opposing results suggest that renal microvessels contain multiple EP receptors. Although all four EP receptors appear to be expressed in the renal microvasculature (23, 31, 37, 43), there is still controversy on this point, because half of these studies failed to find mRNA expression for all four EP receptors (31, 37). The fact that we observed an increase in afferent arteriolar diameter in EP2/+/H11001 mice but a decrease in vessel caliber in EP2/−/− mice in response to PGE2 supports the concept that the renal microcirculation is modulated by multiple PG receptor subtypes.

There is controversy regarding the EP receptor subtype that is responsible for the PGE2-mediated increase in renal blood flow. Recent studies have provided experimental evidence that the EP4 receptor is responsible for the dilator response to PGE2 (31, 37); however, these studies did not directly determine the actions of butaprost on afferent arteriolar diameter or renal blood flow. Interestingly, one of these studies did demonstrate that butaprost opposed the afferent arteriolar constrictor actions of angiotensin by 40% and attributed this response to EP4 receptor stimulation because the study failed to find EP2 mRNA expression in isolated renal microvessels (37). However, this interpretation is at variance with the pharmacological characterization of cloned receptors that suggests that butaprost does not stimulate EP4 receptor-evoked responses at concentrations up to 10 µM (26). Experimental studies in gene-disrupted mice performed by Audoly et al. (3) found that baseline renal blood flow was not different between EP2+/+ and EP2/−/− mice.
and that EP$_2$−/− mice had a vasodilator response to a single dose of PGE$_2$ similar to mice with EP$_2$ receptors. In agreement with these findings, we did not observe a difference in baseline afferent arteriolar diameter between EP$_2$+/+ and EP$_2$−/− mice. On the other hand, we observed a vasoconstriction to PGE$_2$ and butaprost in mice lacking an EP$_2$ receptor. The reason for this difference is unknown. One possible explanation is that the present study investigates afferent arterioles of the juxtamedullary area that give rise to the vasa recta in the medullary circulation. Previous studies have demonstrated that COX inhibition has a greater effect on medullary compared with outer cortical blood flow (11, 14, 32). Other studies also noted differences in responses to PGE$_2$ between superficial and juxtamedullary afferent arterioles (14, 29, 35). The renal vascular distribution of the EP$_2$ receptor and other EP receptors is presently not known. Our studies provide evidence that the EP$_2$ receptor does participate in the renal hemodynamic response to PGE$_2$ and butaprost (Fig. 7).

One very interesting finding of the present study is that afferent arterioles constricted in response to PGE$_2$ and butaprost in mice lacking EP$_2$ receptors. We further demonstrated that angiotensin-converting enzyme inhibition eliminated the afferent arteriolar constrictor response to PGE$_2$ and butaprost in EP$_2$−/− mice. Butaprost-mediated stimulation of the renin-angiotensin system may be due to prostaglandin I$_2$ (IP) receptor activation because butaprost activates IP receptors at micromolar concentrations (22). Butaprost activation of IP receptors is consistent with the observation that prostacyclin is a mediator of COX-dependent renin release (41). These findings do not preclude the possibility that EP$_4$ receptors participate in PGE$_2$-evoked renin release as well. In contrast to the effects of butaprost, Tang et al. (37) demonstrated that the EP$_4$ receptor agonist 11-deoxy-PGE$_1$ completely reversed the afferent arteriolar vasoconstriction to angiotensin II, suggesting EP$_4$ receptor activity opposes the renin-angiotensin system (37). It is important to note that these experiments were conducted in the hydronephrotic kidney that lacks tubules and interactions among the macula densa, juxtaglomerular apparatus, and vascular smooth muscle. Therefore, prostaglandin activation of the renin-angiotensin system would not occur under this experimental setting. Nevertheless, we did observe pregloMERular vasodilation in response to PGE$_2$ during angiotensin-converting enzyme inhibition in EP$_2$−/− mice. This finding supports the concept that EP$_4$ receptors on pregloMERular vessels participate in maintaining renal blood flow.

As mentioned above, butaprost is reported to be an EP$_2$-selective agonist and should not have influenced renal microvessel caliber in mice lacking EP$_2$ receptors. IP receptor activation and renin release could mediate the butaprost-mediated vasoconstriction. Although butaprost has a much lower affinity for the EP$_4$ receptor (5), butaprost actions on the EP$_4$ receptor may be unmasked in mice lacking EP$_2$ receptors. PGE$_2$ also stimulates renal renin release (13, 20), and the EP$_4$ receptor is presently the best candidate for mediating this response (5). PGE$_2$ stimulates cAMP and renin release from juxtaglomerular cells, and intrarenal renin mRNA is not different between wild-type and EP$_2$−/− mice (38). In addition, EP$_4$ but not EP$_2$ receptors are abundantly expressed in glomeruli (6, 9, 36). Thus the results of the present study support the concept that PGE$_2$ activation of the renin-angiotensin system opposes the PGE$_2$-mediated increase in renal blood flow and is not EP$_2$ receptor mediated and may be mediated by EP$_4$ and/or IP receptor activation (Fig. 7). We cannot rule out the possibility that activation of other vasoactive pathways might participate in the PGE$_2$-mediated afferent arteriolar vasoconstriction observed in EP$_2$−/− mice.

The afferent arteriolar response to sulprostone was assessed in mice lacking EP$_2$ receptors to determine whether EP$_2$ receptors opposed EP$_1$ and EP$_3$ receptor-mediated vasoconstriction. Sulprostone decreased afferent arteriolar diameter to a greater extent in mice lacking EP$_2$ receptors compared with EP$_2$+/+ mice. This finding confirms that EP$_1$ or EP$_3$ receptor activation results in an increase in renal vascular resistance (Fig. 7) (5, 31, 37). There is still controversy regarding which EP receptor is responsible for the vasoconstrictor response to PGE$_2$. A recent study demonstrated afferent arteriolar constriction in response to the EP$_1$−/− selective agonist sulprostone, but this response was not blocked by the EP$_1$ antagonist SC-51322 (37). These findings suggest that the EP$_3$ receptor is primarily responsible for the renal vasoconstrictor response to PGE$_2$. In contrast, evidence for EP$_1$ but not EP$_3$ receptors in rat pregloMERular vessels has recently been demonstrated (31). Purdy and Arendshorst (31) did not observe inhibition of isoproterenol elevation of cAMP levels in renal microvessels by the EP$_3$ agonist M&B28767. Interestingly, this same group has data that suggest that EP$_3$ receptors are important to the
control of renal hemodynamics in the mouse (3). Renal blood flow was elevated and the vasodilatory response to PGE2 was enhanced in mice that lack the EP3 receptor. Additionally, systemic administration of the EP3 agonist SC-46275 resulted in a prolonged elevation of arterial blood pressure in mice lacking EP2 receptors (43). The results of the present study demonstrate that EP3 receptors oppose the preglomerular vasoconstrictor response to sulprostone.

The contribution of endothelin-1 to the development of salt-sensitive hypertension is well established (30, 34). Interestingly, mice that lack EP2 receptors develop hypertension when fed a high-salt diet (21). Therefore, we investigated the contribution of EP2 receptors to oppose the afferent arteriolar vasoconstrictor response to endothelin-1. Afferent arterioles from EP2−/− mice were more responsive to endothelin-1 compared with EP2+/+ mice. Along these lines, Oyekan and McGiff (28) demonstrated that the endothelin-1-evoked decreases in renal blood flow and glomerular filtration were enhanced by COX inhibition. In contrast, indo- methacin attenuated the increase in renal vascular resistance, the afferent arteriolar decrease in diameter, and renal microvascular smooth muscle cell calcium response to endothelin-1 (18, 27). The results of the present study also suggest involvement of COX-derived vasodilator and vasoconstrictor metabolites in the afferent arteriolar response to endothelin-1. The enhanced response to endothelin-1 observed in mice lacking EP2 receptors was eliminated by COX inhibition. Thus PGE2 activation of EP2 receptors and the resultant vasorelaxation oppose COX-mediated renal vasoconstrictor mechanisms in response to endothelin-1.

In summary, afferent arteriolar diameter of EP2+/+ increased in response to PGE2 and butaprost, whereas PGE2 and butaprost decreased the diameter of afferent arterioles in EP2−/− mice. The renal vasoconstriction to butaprost in EP2−/− mice was eliminated by enalapril. This observation supports the concept that the renin-angiotensin system contributed to the PGE2-mediated vasoconstriction in EP2−/− mice. Mice lacking EP2 receptors also exhibited a greater vasoconstriction to the EP1 and EP3 agonist sulprostone. Endothelin-1 elicited a greater afferent arteriolar vasoconstrictor response in mice lacking EP2 receptors. COX inhibition ameliorated this enhanced endothelin-1 response in EP2−/− mice. Overall, these studies support the concept that EP2 receptors participate in the maintenance of afferent arteriolar function.

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