Stimulation of renin release by prostaglandin E$_2$ is mediated by EP$_2$ and EP$_4$ receptors in mouse kidneys

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Schweda, Frank, Jürgen Klar, Shuh Narumiya, Rolf M. Nüsing, and Armin Kurtz. Stimulation of renin release by prostaglandin E$_2$ is mediated by EP$_2$ and EP$_4$ receptors in mouse kidneys. Am J Physiol Renal Physiol 287: F427–F433, 2004. First published April 27, 2004; 10.1152/ajprenal.00072.2004.—PGE$_2$ is a potent stimulator of renin release. So far, the contribution of each of the four PGE$_2$ receptor subtypes (EP$_1$–EP$_4$) in the regulation of renin release has not been characterized. Therefore, we investigated the effects PGE$_2$ on renin secretion rates (RSR) from isolated, perfused kidneys of EP$_1$−/−, EP$_2$−/−, EP$_3$−/−, and wild-type mice. PGE$_2$ concentration dependently stimulated RSR from kidneys of all four knockout strains with a threshold concentration of 1 nM in EP$_1$−/−, EP$_2$−/−, EP$_3$−/−, and wild-type mice, whereas the threshold concentration was shifted to 10 nM in EP$_4$−/− mice. Moreover, the maximum stimulation of RSR by PGE$_2$ at 1 μM was significantly reduced in EP$_4$−/− (12.8-fold of control) and EP$_3$−/− (15.9-fold) compared with wild-type (20.7-fold), EP$_1$−/− (23.8-fold), and EP$_2$−/− (20.1-fold). In contrast, stimulation of RSR by either the loop diuretic bumetanide or the β-adrenoceptor agonist isoproterenol was similar in all strains. PGE$_2$ exerted a dual effect on renal vascular tone, inducing vasodilatation at low concentrations (1 nmol/l) and vasoconstriction at higher concentrations (100 nmol/l) in kidneys of wild-type mice. In kidneys of EP$_2$−/− as well as EP$_4$−/− mice, vasodilatation at low PGE$_2$ concentrations was prevented, whereas vasoconstriction at higher concentrations was augmented. In contrast, the vasodilatatory component was pronounced in kidneys of EP$_1$ and EP$_3$ knockout mice, whereas in both genotypes the vasoconstriction at higher PGE$_2$ concentrations was markedly blunted. Our data provide evidence that PGE$_1$ stimulates renin release via activation of EP$_1$ and EP$_4$ receptors, whereas EP$_1$ and EP$_3$ receptors appear to be without functional relevance in juxtaglomerular cells. In contrast, all four receptor subtypes are involved in the control of renal vascular tone, EP$_1$ and EP$_3$ receptors increasing, and EP$_2$ as well as EP$_4$ receptors, decreasing it.

renin secretion; renal vascular resistance; bumetanide; isolated, perfused mouse kidney

PROSTAGLANDINS ARE CRITICAL regulators of kidney function as they impact on renal vascular tone and tubular electrolyte and water excretion (3). The early observation that nonselcetive blockade of the prostanooid-generating cyclooxygenases by nonsteroidal anti-inflammatory drugs can lower plasma renin activity in different species, including humans, indicated that these autacoids stimulate renin release (2, 22, 23). Moreover, the recent discovery of the inducible cyclooxygenase isofrom COX-2 as being expressed constitutively in the thick ascending limb of Henle, including the macula densa cells (11), suggested a specific role for COX-2-derived prostaglandins in the regulation of the renin system. Indeed, several studies performing pharmacological blockade or genetic deletion of COX-2 reported an inhibition of renin release and renin expression by these maneuvers (4, 6, 30, 33, 35). Besides this indirect evidence of a role for prostaglandins in the regulation of the renin system, several investigations confirmed that both PGE$_2$ as well as PGI$_2$ stimulate renin release. Thus these prostanooids enhanced renin secretion in dogs in vivo (8, 32), in isolated afferent arterioles (16), renal cortical slices (7, 13, 19, 34), and isolated, perfused kidneys of rats (10). Furthermore, PGI$_2$ and PGE$_2$ enhanced renin release as well as renin mRNA expression in primary cultures of mouse juxtaglomerular cells, indicating that the effect of PGE$_2$ on the renin system is a direct cellular effect (17). Because both prostanooids not only stimulated renin release but also cAMP accumulation in the juxtaglomerular cell preparation and cAMP is known to be a critical intracellular signal stimulating the exocytosis of renin, it was concluded that the stimulation of the renin system by PGI$_2$ and PGE$_2$ is mediated by stimulation of adenylyl cyclase activity (17).

In general, PGE$_2$ exerts its biological actions via four G protein-coupled receptor subtypes, PGE$_{1,4}$ receptors (EP$_{1,4}$) (3). Whereas EP$_2$ and EP$_3$ receptors activate adenylyl cyclase, resulting in intracellular cAMP accumulation, the EP$_1$ receptor has the opposite effect as it is negatively coupled to adenylyl cyclase (3). The EP$_1$ receptor signals via increasing the intracellular calcium concentration and stimulating inositol 1,4,5-trisphosphate generation (3). Noteworthy, the intracellular second messengers cAMP and calcium are considered to be the main regulators of renin release, with cAMP stimulating the exocytosis of renin and an increase in intracellular calcium concentration inhibiting it (9). Therefore, in principle all four EP receptor subtypes might be involved in the regulation of renin release by PGE$_2$, the EP$_2$ and EP$_3$ receptors stimulating, and the EP$_1$ and EP$_3$ receptors inhibiting, renin secretion. Interestingly, such a dual effect of PGE$_2$ has been reported by previous studies investigating the effects of PGE$_2$ on renal vascular tone. Thus PGE$_2$ induced vasodilatation by either EP$_2$ or EP$_3$ receptor activation, and this decrease in vascular resistance was counteracted by coactivation of the EP$_1$ or EP$_3$ receptors, depending on the species. PGE$_2$ concentration applied, and experimental approach (1, 15, 21, 28). The fact that the renin-producing juxtaglomerular cells are transformed vascular smooth muscle cells and therefore share several charac-

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teristics with these cells (9) makes it reasonable to assume that PGE2 exerts a dual effect not only on renal vascular contractility but also on renin secretion. To test this hypothesis, we compared the effects of PGE2 on the renin secretion rates from isolated, perfused kidneys of mice with a genetic deletion of the EP1, EP2, EP3, or EP4 receptor and their respective wild-type controls. Moreover, as COX-2-derived prostaglandins have been suggested to mediate macula densa control of renin release (30), we investigated the stimulation of renin secretion by loop diuretics, which is at least partially mediated by the macula densa, in isolated, perfused kidneys of EP knockout mice.

Finally, as the isolated, perfused mouse kidney model allows not only the determination of renin secretion rates but also renal vascular resistance (RVR), the contribution of each of the four EP receptor subtypes in the regulation of RVR by PGE2 in mice was studied under controlled conditions.

MATERIALS AND METHODS

**EP receptor knockout mice.** EP1−/−, EP2−/−, EP3−/−, and EP4−/−deficient mice were generated as previously described (14, 26, 31, 37). All strains investigated were in the mixed genetic background of 129/Ola × C57BL/6. The mice were weaned at 3 wk of age and fed a standard chow diet. They were housed under controlled conditions (temperature 21 ± 1°C, 12:12-h light-dark cycle). Genotypes of the mice were routinely determined by PCR analysis using oligonucleotide primers designed to detect the respective EP locus and Neo cassette, as reported earlier (14, 26, 31).

All experiments were conducted in accordance with the Institute for Laboratory Research Guide for the Care and Use of Laboratory Animals (Washington, DC: National Academy Press, 1996) and German laws on the protection of animals.

**Isolated, perfused mouse kidney.** Male mice (22–27 g body wt) were used as kidney donors. The animals were anesthetized with an intraperitoneal injection of 100 mg/kg 5-ethyl-5-(1-methylbutyl)-2-thiobarbituric acid (Byk Gulden) and 80 mg/kg ketamine-HCl (Curamed). After the abdominal cavity was opened, 50 μl of blood were taken by aortic puncture for the determination of plasma renin concentration (PRC). After centrifugation, the plasma was stored at −20°C until further processing. Subsequently, the aorta was cannulated with the perfusion cannula, the right kidney was excised, and finally the renal vein was cannulated according to the technique described previously (25). The venous effluent was drained outside the moistening chamber and collected for determination of renin activity and venous blood flow. Kidneys were perfused at a constant pressure of 100 mmHg.

The basic perfusion medium, supplied from a thermostated (37°C) reservoir of 200 ml, consisted of a modified Krebs-Henseleit solution containing (in mM) all physiological amino acids in concentrations between 0.2 and 2.0, 8.7 glucose, 0.3 pyruvate, 2.0 l-lactate, 1.0 α-ketoglutarate, 1.0 l-malate, and 6.0 urea, as well as ampicillin (3 mg/100 ml) and fluocarcillin (3 mg/100 ml). The perfusate was supplemented with 6 g/100 ml bovine serum albumin, 1 mM/100 ml vasopressin 8-lysine, and freshly washed human red blood cells (10% hematocrit). To improve the functional preservation of the preparation, the perfusate was continuously dialyzed against a 10-fold volume of the same composition, but lacking erythrocytes and albumin. For oxygenation of the perfusion medium, the dialysate was gassed with a 94% O2-6% CO2 mixture.

Perfusate flow was calculated by collection and gravimetric determination of the venous effluent. Perfusion pressure was continuously monitored by a potentiometric recorder.

After constant perfusion pressure was established, perfusate flow rates usually stabilized within 15 min. Stock solutions of PGE2 (Cayman Chemical) and CAY 10399 (Cayman Chemical) were added to the perfusate; a stock solution of bumetanide (Sigma) was added to the dialysate.

**Determination of perfusate renin activity and PRC.** For determination of perfusate renin activity, the venous effluent from isolated kidneys was collected over a period of 30 s at intervals of 2 min in the PGE2 dose-response experiments and at intervals of 2.5 min in the bumetanide experiments. The samples were centrifuged at 1,500 g for 10 min, and the supernatants were stored at −20°C until assayed for renin activity. For determination of renin activity, the perfusate samples were incubated for 1.5 h at 37°C with plasma from bilaterally nephrectomized male rats as the renin substrate. The generated ANG I (ng·ml−1·h−1) was determined by radioimmunoassay (Byk & Dia-Sorin Diagnostics). Renin secretion rates were calculated as the product of renin activity and venous flow rate (ml·min−1·g kidney wt−1).

PRC of EP−/− mice and their respective wild-type controls was determined in blood samples obtained before insertion of the perfusion cannula into anesthetized mice. Plasma was incubated with excess renin substrate, and the generated ANG I was determined by RIA (Byk & Dia-Sorin Diagnostics).

**Statistical analysis.** Values are given as means ± SE. For statistical analysis, the two values obtained within an experimental period were averaged. Student’s paired t-test was used to calculate levels of significance within individual kidneys. Differences between groups were analyzed by ANOVA and Bonferroni’s adjustment for multiple comparisons. P values <0.05 were considered statistically significant.

**RESULTS**

**Role of EP receptors in the regulation of renin secretion.** Because the renin secretion rates of the wild-type kidneys of the respective knockout strains (n = 3/strain) did not differ under baseline or under stimulated conditions, the results from wild-type mice were combined.

As shown in Figs. 1 and 2, there was no detectable difference among the renin secretion rates of kidneys of EP1−/−, EP2−/−, EP3−/−, and EP4−/− mice or their wild-type controls under baseline conditions.

PGE2 stimulated renin secretion rates from isolated kidneys of wild-type mice in a concentration-dependent fashion, starting at a concentration of 1 nmol/l PGE2 (2.8-fold of baseline) and reaching a plateau at 1 μmol/l (20.6-fold of baseline) (Figs. 1 and 2).

PGE2-induced renin release was significantly attenuated in kidneys of EP2 as well as EP4 receptor knockout mice. Although PGE2 stimulated renin release in the kidneys of both knockout strains, the renin secretion rates of kidneys from EP2−/− and EP4−/− mice were reduced compared with their wild-type controls at several concentrations of PGE2 (Figs. 1 and 2). Thus, although the threshold concentration was 1 nmol/l in kidneys of EP2−/− mice as well as in wild-type kidneys, half-maximal stimulation was shifted to a concentration of 26 nmol/l PGE2 in EP2−/− (wild-type 7.5 nmol/l), and maximal stimulation at 1 μmol/l was significantly reduced to 653 ± 145 ng ANG I·h−1·min−1·g−1 compared with wild-type mice (1,206 ± 117 ng ANG I·h−1·min−1·g−1) (Figs. 1 and 2). In kidneys of EP4 receptor knockout mice, the stimulation of renin release by PGE2 was attenuated over the entire concentration range tested. Thus renin secretion was not stimulated by a concentration of 1 nmol/l of PGE2, whereas this concentration significantly enhanced renin release from kidneys of EP1, EP2, and EP4 knockout and wild-type mice. Instead, in kidneys of EP4−/−, the threshold concentration was shifted to 10 nmol/l, half-maximal stimulation oc-
release by PGE$_2$, subsequent activation of EP receptors (Fig. 2), and concomitant administration of isoproterenol (10 nmol/l at 1,000 nmol/l) – EP$_2$–/− and EP$_3$–/− mice (Fig. 3). The finding that PGE$_2$ caused enhancement of renin release was reduced in kidneys of EP$_2$ and EP$_4$ receptor knockout mice led to the conclusion that both receptors mediate the stimulatory effects of PGE$_2$. Therefore, we next tested whether direct pharmacological activation of these receptors stimulated renin secretion rates from kidneys of wild-type mice. Indeed, activation of EP$_2$ receptors using the specific EP$_2$ agonist CAY 10399 (1 μmol/l) (29) stimulated renin secretion significantly to 3.8 ± 0.9-fold of control in wild-type mice, whereas it was without effect on renin release in EP$_2$–/− mice (Fig. 3). Subsequent administration of PGE$_2$ further stimulated renin release in wild-type mice to 12.0 ± 2.8-fold of baseline and increased renin secretion to 7.6-fold in EP$_2$–/− mice (Fig. 3).

To define whether the observed in vitro results were paralleled by accordant in vivo findings, we determined PRC in anesthetized EP receptor knockout mice. As shown in Fig. 4, PRC was significantly reduced in EP$_4$–/− mice compared with their wild-type controls. No significant differences of PRC were detected between EP$_1$–/−, EP$_2$–/−, EP$_3$–/− mice and their respective wild-type controls (Fig. 4).

Because our data, derived from experiments performing direct activation of EP receptors, clearly demonstrate that both EP$_2$ as well as EP$_4$ receptors mediate the PGE$_2$ stimulation of renin release, we wondered whether these receptors are involved in the more functional regulation of renin release as well. Because several lines of evidence suggest that prostaglandins mediate macula densa stimulation of renin release (6, 11, 30, 33, 35), we studied the specific role of the EP receptor subtypes in the macula densa mechanism in the next set of experiments. To this end, we blocked thick ascending limb and macula densa salt transport using the loop diuretic bumetanide in isolated, perfused kidneys of EP knockout mice. As shown in Fig. 5, bumetanide (100 μmol/l) doubled renin secretion by isoproterenol (10 nmol/l) further enhanced renin secretion rates to similar levels in all strains (Fig. 2).

Fig. 1. Effects of PGE$_2$ (0.1–1,000 nmol/l) on renin secretion rates from isolated, perfused kidneys of EP$_1$–/−, EP$_2$–/−, EP$_3$–/−, EP$_4$–/− knockout mice (n = 4/strain) and their wild-type controls (WT; n = 3/genotype). Values are means ± SE. As wild-type controls did not differ regarding their renin secretion rates, their results were combined. For statistical analysis, see the text and Fig. 2.

Subsequent administration of PGE$_2$ further stimulated renin release in wild-type mice to 12.0 ± 2.8-fold of baseline and increased renin secretion to 7.6-fold in EP$_2$–/− mice (Fig. 3). Despite the marked differences in the stimulation of renin secretion rates, their results were combined. For statistical analysis, see the text and Fig. 2.

Fig. 2. Renin secretion rates from isolated, perfused kidneys of EP$_1$–/−, EP$_2$–/−, EP$_3$–/−, EP$_4$–/− mice, and their wild-type controls. Values are means ± SE. The stepwise increase in PGE$_2$ concentration (0.1–1,000 nmol/l) and concomitant administration of isoproterenol (10 nmol/l at 1,000 nmol/l PGE$_2$) are shown. For statistical analysis, the last 2 values of a respective experimental period were averaged. *P < 0.05 vs. control. **P < 0.05 vs. WT.

Fig. 3. Effects of selective stimulation of EP$_2$ receptors by CAY 10399 (1 μmol/l) and subsequent administration of PGE$_2$ (1 μmol/l) on renin secretion rates from isolated, perfused kidneys of WT or EP$_2$–/− mice. Values are means ± SE.
rates in wild-type as well as in all four knockout strains, without any significant differences among the strains (Fig. 5).

**Effects of PGE\(_2\) on renal vascular tone of EP receptor knockout mice.** In contrast to the effects of PGE\(_2\) on renin release that are stimulatory over the complete concentration range from 1 nmol/l to 1 μmol/l, PGE\(_2\) exerted a biphasic effect on RVR. As demonstrated in Fig. 6, 1 nmol/l PGE\(_2\) significantly lowered RVR compared with control values, whereas higher concentrations (100 nmol/l, 1 μmol/l) increased RVR in wild-type mice. This pattern was markedly altered in each of the four knockout strains. In kidneys of EP\(_1/-/-\) as well as of EP\(_3/-/-\) mice, the vasodilatory effects of PGE\(_2\) were preserved over a wider concentration range, and even PGE\(_2\) (1 μmol/l) did not significantly increase RVR (Fig. 6). In contrast, PGE\(_2\) did not induce vasodilatation in kidneys of EP\(_2/-/-\) or EP\(_4/-/-\) mice but enhanced RVR in kidneys of EP\(_4/-/-\) mice, already at a concentration of 1 nmol/l, a concentration that decreased RVR in kidneys of wild-type, EP\(_1/-/-\), and EP\(_3/-/-\) mice.

**DISCUSSION**

Although it has long been known that PGE\(_2\) influences renin release (32), the receptor subtypes being involved in this regulation have not been identified so far. Therefore, the primary aim of our study was to characterize the specific role of each of the four known PGE\(_2\) receptors in the regulation of renin release by PGE\(_2\).

We demonstrate that PGE\(_2\) potently stimulates renin release from isolated mouse kidneys. Moreover, as the increase of the renin secretion rates was not completely abrogated in any of the knockout strains, the data clearly indicate that PGE\(_2\) does not exert its stimulatory effects on the renin system exclusively via activation of one single receptor. Instead, both EP\(_2\) and EP\(_4\) receptors act in concert to achieve full stimulation of renin release by PGE\(_2\), because both the half-maximal stimulation of renin release occurred at higher PGE\(_2\) concentrations and the maximum renin secretion rate was reduced in kidneys of both EP\(_2/-/-\) and EP\(_4/-/-\) mice. This attenuation of PGE\(_2\)-induced renin release was not caused by a general impairment of the capacity to secrete renin in kidneys of EP\(_2\) or EP\(_4\) knockout mice, as stimulation of renin release by β-adrenoreceptor activation was completely preserved in both strains. The functional role of the EP\(_2\) receptor in the regulation of renin release by PGE\(_2\) was further underlined by the finding that selective EP\(_2\) receptor activation by CAY 10399 (1 μmol/l) significantly stimulated renin release in wild-type mice. At this maximum effective concentration of CAY 10399, the effect on renin release was selectively mediated by activation of EP\(_2\) receptors, as CAY 10399 (1 μmol/l) did not stimulate renin secretion in kidneys of EP\(_2/-/-\) mice. Even in this setting of maximum preactivation of EP\(_2\) receptors by CAY 10399, renin secretion rates were further enhanced by subsequent adminis-
tration of PGE2, suggesting this additional stimulation was mediated by EP4 receptors and further underlining the conclusion that both the EP2 and the EP3 receptor mediate the PGE2-induced stimulation of renin release. Interestingly, the threshold concentration of PGE2 was shifted one order in EP2−/− mice (1 nmol/l PGE2 did not stimulate renin secretion in kidneys of EP4−/− mice), whereas this concentration was effective in wild-type mice and all other genotypes including EP3−/−. These data suggest that EP2 receptors might be responsible for the stimulation of the renin system at low concentrations of PGE2. At this point, it is reasonable to consider the concentrations of PGE2 that occur in the area of the juxtaglomerular cells in vivo. Although PGE2 concentration at the juxtaglomerular cell site has not been determined directly, a concentration of ~3–10 nmol/l has been measured in the tubular fluid of early proximal tubules of rats (24). Because PGE2 is not synthesized in proximal tubules and secretion of PGE2 is minor (24), the PGE2 concentration in the proximal tubule mirrors the PGE2 content in the juxtaglomerular area that has been filtered in the glomerulus. Given that a similar concentration of PGE2 occurs in kidneys of mice, our in vitro data would suggest that the EP2 receptor is critical for the stimulation of the renin system by PGE2 under normal conditions. In fact, PRC was significantly lower in EP2−/− mice compared with their wild-type controls, compatible with the conclusion that EP2 receptors are of functional relevance for the stimulation of the renin system in vivo. In contrast to EP2−/− mice, EP2−/− mice had a normal PRC in our study, in line with a previous report that demonstrated normal plasma renin activity and renin expression in EP2−/− mice (5). At first glance, these results appear somewhat at odds with our in vitro data. However, a putative suppression of the renin system by genetic deletion of the EP2 receptor might be counterregulated by systemic factors, for example, renal nerve activity. Moreover, as indicated by our in vitro data, EP2 receptors appear not to be critical for the stimulation of renin release at low concentrations of PGE2 and therefore might not be involved in the regulation of PRA under normal conditions but in situations of markedly enhanced prostanoid formation, as with inflammation, for example. Further studies are needed to test this hypothesis.

Because activation of EP1 and EP3 receptors has been demonstrated to counterregulate the renal vasodilatation mediated by EP2 and EP4 receptors and the renin-producing juxtaglomerular cells share several similarities with vascular smooth muscle cells (9), we had hypothesized that EP1 and EP3 receptors might also buffer the stimulation of renin release by PGE2. If this hypothesis were true, one would expect that genetic deletion of EP1 or EP3 receptors should result in an enhanced stimulation of renin release by PGE2. However, we could not detect any difference in the renin secretion rates between the kidneys of EP1−/− or EP3−/− mice compared with their wild-type controls, arguing against an involvement of these receptors in the regulation of the renin system. Although we did not find any statistical significance difference in PRC between EP1−/− and EP3−/− mice and their respective wild-type controls, there was a trend to higher PRC values in both knockout strains. In EP1−/− mice, elevated plasma renin activity has been reported previously (27) that, however, had been suggested to be a secondary effect of the reduced arterial blood pressure in these mice.

In a second series of experiments, we investigated the role of the respective EP receptor subtypes in the macula densa control of renin release. Providing the rationale for these studies were several previous examinations, those performing either genetic deletion or pharmacological blockade of prostanoid-forming COX-2, that strongly suggested prostaglandins to be critical mediators of the macula densa mechanism (6, 30, 35). Moreover, macula densa cells have been demonstrated to release PGE2 in a salt-dependent fashion, a low extracellular or tubular NaCl concentration stimulating PGE2 release and a higher concentration inhibiting it (20, 36). Therefore, it is reasonable to assume that PGE2 released by macula densa cells in response to a low NaCl concentration stimulates renin release. Stimulation of renin secretion by the macula densa mechanism can be achieved experimentally by blockade of tubular and macula densa salt transport using loop diuretics. As the salt transport rate of NKCC2 is a critical step in the detection of the tubular salt concentration by macula densa cells (12), blockade of this transporter somewhat mimics a low tubular salt concentration and, consequently, stimulates renin release. Indeed, the loop diuretic bumetanide stimulated renin secretion rates to twofold of control in isolated, perfused kidneys of wild-type mice in the present study, as we have reported previously (25). However, we could not detect any differences in the stimulation of renin release by bumetanide between the kidneys of the EP knockout strains and their wild-type controls, arguing against an involvement of EP receptors in the macula densa control of renin release. Therefore, in concert with the above-mentioned studies suggesting an involvement of COX-2 in the macula densa stimulation of renin release, our data give rise to the speculation that it is not PGE2 but PGI2, also a potent direct stimulator of renin release (17), which is the critical regulator of salt-dependent renin release; this should be the subject of future study. However, the possibility that other factors besides the macula densa mechanism might be involved in the stimulation of renin release by loop diuretics should also be taken into account. This hypothesis is in line with previous studies demonstrating that blockade of COX-2 in rats was capable of reducing but not completely blunting the stimulation of plasma renin activity by furosemide, although formation of prostanoids was reduced to baseline levels (18).

Besides the role of the EP receptors in the PGE2-induced stimulation of renin release, we determined their effects on renal vascular tone of isolated, perfused mouse kidneys. PGE2 exerted dual effects on RVR in wild-type mice, as it induced vasodilatation at low concentrations but led to a marked vasoconstriction at higher concentrations. This finding is completely in line with previous elegant studies in isolated, perfused hydronephrotic kidneys of rats that reported PGE2 as increasing the vessel diameter of afferent arterioles at a concentration of 10 nmol/l and to decrease it at 1 μmol/l (28).

In general, all four EP receptor subtypes might be involved in the regulation of vascular tone by PGE2: EP2 and EP4 receptors stimulating cAMP generation and thereby inducing vasodilatation and EP1 and EP3 receptors increasing the intracellular calcium concentration or inhibiting cAMP formation, resulting in vasoconstriction. Indeed, all four receptors have been suggested to be involved in the effects of PGE2 on renal vascular contractility. However, those previous investigations of the contribution of the respective EP receptors in the regulation of renal vascular tone of mice and rats are somewhat
unequivocal. For instance, in rats the responsibility for renal vasconstriction has been attributed to both EP₁ (21) and EP₄ receptors (28), whereas both of the aforementioned studies identified the EP₁ receptor as mediating vasodilatation. In one study in mice, vasodilatation of afferent arterioles caused by PGE₂ was primarily related to an effect at EP₂ receptors (15), whereas in another study EP₂ receptors have been reported to be without a specific role in the regulation of overall renal hemodynamics (1). The results of the present study suggest that all four receptors act in concert to regulate overall renal hemodynamics, as vasodilatation due to PGE₂ is blunted in normal conditions at low PGE₂ concentrations. EP₁ as well as EP₃ receptors appear to be without functional relevance in juxtaglomerular cells. In contrast, all four EP receptors are relevant to the regulation of renal vascular tone by PGE₂, with EP₁ and EP₃ receptors inducing vasoconstriction and EP₂ and EP₄ receptors mediating vasodilatation in the renal vasculature.

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