EP₁ and EP₄ receptors mediate prostaglandin E₂ actions in the microcirculation of rat kidney

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Purdy, Kit E., and William J. Arendshorst. EP₁ and EP₄ receptors mediate prostaglandin E₂ actions in the microcirculation of rat kidney. *Am J Physiol Renal Physiol* 279: F755–F764, 2000.—Vasodilator prostaglandin PGE₂ protects the kidney from excessive vasoconstriction during contraction of extracellular fluid volume and pathophysiological states. However, it is not yet clear which of the four known E-prostanoid (EP) receptors is localized to resistance vessels and mediates net vasodilation. In the present study, we assessed the presence, signal transduction, and actions of EP receptor subtypes in pregglomerular arterioles of Sprague-Dawley rat kidneys. RNA encoding EP₁, an EP₁-variant, and EP₄ receptors was identified by RT-PCR in freshly isolated pregglomerular microvessels; cultured pregglomerular vascular smooth muscle cells (VSMC) had EP₁-variant and EP₄ RNA but lacked EP₁. EP₂ and EP₃ receptors were undetectable in both vascular preparations. In studies of cell signaling, stimulation of cAMP by various receptor agonists is consistent with primary actions of PGE₂ on the EP₄ receptor, with no inhibition of cAMP by EP₁ receptors. Studies of cytosolic calcium concentration in cultured renal VSMC support an inhibitory role of EP₄ during ANG II stimulation. In vivo renal blood flow (RBF) studies indicate that the EP₄ receptor is the primary receptor mediating sustained renal vasodilation produced by PGE₂, whereas the EP₁ receptor elicits transient vasoconstriction. The EP₁-variant receptor does not appear to possess any cAMP or cytosolic calcium signaling capable of affecting RBF. Collectively, these studies demonstrate that the EP₄ receptor is the major receptor in pregglomerular VSMC. EP₄ mediates PGE₂-induced vasodilation in the rat kidney and signals through Gₛ proteins to stimulate cAMP and inhibit cytosolic calcium concentration.

The paracrine agent PGE₂ is the predominant cyclooxygenase metabolite of arachidonic acid in the kidney (9). PGE₂ plays an important role in tubular reabsorption of salt and water as well as in the control of renal vascular resistance and the maintenance of glomerular hemodynamics. Vasoconstrictor hormones such as ANG II and norepinephrine, which are released during periods of effective volume depletion, stimulate PGE₂ production in many vascular beds, including the kidney. Despite several reports of PGE₂-induced vasoconstriction (5, 20), there is convincing evidence that the primary action of PGE₂ is to counteract the effects of these pressor hormones and protect the kidney from excessive vasoconstriction. Injection of small amounts of PGE₂ directly into the renal artery causes vasodilation in vivo (12, 22), and infusion of PGE₂ into the renal artery effectively attenuates hormone-induced vasoconstriction (11, 25). Conversely, blockade of prostaglandin production enhances vasoconstrictor activity (1, 17). PGE₂ appears to act primarily on the pregglomerular vasculature to decrease vascular resistance (2, 18). It has been shown to buffer ANG II-induced constriction in isolated renal arterioles (16, 38) and buffer ANG II-induced increases in cytosolic calcium in vascular smooth muscle cells (VSMC) isolated from the pregglomerular vasculature (32). Little is known about the receptor subtype initiating and cellular signaling events underlying PGE₂ action in the renal vasculature.

In general, the actions of PGE₂ are mediated by a family of G protein-coupled receptors. Four E-prostanoid (EP) receptors, termed EP₁ through EP₄, were originally identified pharmacologically and then further characterized by cloning (14, 30, 31, 37). Transfection of recombinant receptors into host cells, such as Chinese hamster ovary or human embryonic kidney cells, has revealed that each receptor couples to a different signal transduction pathway. In these noncontractile cells, the EP₁ receptor activates Gₛ proteins to elevate intracellular calcium (31); EP₂ and EP₄ stimulate cAMP production via Gₛ (8, 30); and EP₃ inhibits cAMP formation via Gₛ (8). Alternatively, splice isoforms have been identified for both EP₁ and EP₄, that may couple to various signaling pathways. Whether these same second messenger systems are coupled to their respective EP receptor subtypes in natural cells remains to be established.

Although the role of individual EP receptors in the renal vasculature is not known, expression and func-
tion of EP receptor subtypes in the distal nephron have been characterized. The EP$_1$ receptor, detected in the collecting duct of humans (10, 28) and mice (35), is reported to mediate PGE$_2$ inhibition of sodium reabsorption (19). EP$_3$ is present in the thick ascending limb and collecting duct of humans (10, 28), mice (29), and rats (36) and also appears to inhibit salt and water reabsorption.

Recent studies of EP receptor distribution in rat renal cells other than epithelial have focused on mRNA expression in the glomerulus, more than on renal arteries and arterioles. Ribonuclease protection assays demonstrated predominant expression of EP$_1$ in rat glomeruli with lower levels of EP$_1$ and EP$_3$ (23). In cell culture models, gene expression of EP$_1$ and EP$_4$ receptors is noted in rat mesangial cells (21) and podocytes (7). Little is known about the function and cellular actions of EP receptor types in resistance vessels in general and the renal microcirculation in particular. Whether findings for glomeruli and cultured mesangial cells correlate with message, protein expression, and functional effects in the preglomerular arterioles is not known.

The purpose of the present study was to determine which EP receptors are expressed in preglomerular resistance vessels and, of these, which subtypes act to mediate vascular actions of PGE$_2$. RT-PCR was used to identify the presence of receptor mRNA in both freshly isolated preglomerular microvessels and cultured preglomerular VSMC. Cell signaling via EP receptors was assessed in vitro by measuring cAMP production using radioimmunoassay and by measuring changes in cytosolic calcium concentration using the fluorescent dye fura 2. In vivo renal blood flow (RBF) studies evaluated renal vascular reactivity to PGE$_2$ injected into the renal artery. The contribution of the different EP receptors in renal resistance vessels was further defined using pharmacological receptor agonists and antagonists in cell signaling and RBF experiments.

**METHODS**

Isolation of preglomerular resistance vessels. Renal preglomerular arterioles were isolated from kidneys of 6-wk-old male Sprague-Dawley rats from our Chapel Hill breeding colony (originally derived from Harlan, Indianapolis, IN) by use of previously established methodology (32, 40). Approximately three rats were used for each preparation of isolated VSMC. Sterile solutions and equipment were used throughout. Briefly, the kidneys were infused with a magnetized iron oxide suspension (1% Fe$_3$O$_4$ in phosphate-buffered saline), followed by brief centrifugation and suspended in culture medium [(in mM) 50 HEPES, 8 MgCl$_2$, IBMX, 2 ATP, 4 GTP, and 60 phosphocreatine as well as 800 µg/ml creatine phosphokinase] and buffer B [(in mM) 50 HEPES, 1 EDTA, 1 dithiothreitol, and 0.25 sucrose as well as 1 mg/ml BSA] to give a final concentration of 50 µg protein/ml. This mixture was incubated for 15 min at 37°C with ANG II (Sigma), PGE$_2$ (Cayman Chemical), isoproterenol (Sigma), or various EP receptor agonists (suprostone, Draken, 17-phenyl-PGE$_2$ (Cayman Chemical), butaprost (Bayer), misoprostol (Biomol), and M&B28767 (Rhone-Poulenc Rorer)). In another set of experiments, fresh VSMC were pretreated with EP receptor antagonists [SC-19220 (Searle), AH-23848 (Glaxo-Wellcome), or AH-6809 (Biomol)] for 5 min before PGE$_2$ stimulation for 15 min. The reaction was stopped by adding TCA to produce a 6% final concentration. The samples were immediately put on ice and sonicated for 1 min. The cell lysate was extracted four times with 1 ml of water-saturated ether and evaporated. Aliquots were acetylated, and cAMP was measured by radioimmunoassay (Biomedical Technologies).

Measurement of cysotolic free calcium concentration. Cytosolic free calcium concentration ([Ca$^{2+}$]$_i$) was measured in cultured VSMC of preglomerular arterioles by using the calcium-sensitive dye fura 2-acetoxymethyl ester (AM) as described previously (32, 40). Confluent VSMC grown on glass coverslips were subjected to serum-free medium 24 h before an experiment. Before the study, monolayers of VSMC were washed twice with Hanks’ balanced salt solution (HBSS; (in mM) 135 NaCl, 5 KCl, 1 CaCl$_2$, 1 MgCl$_2$, 5
D-glucose, and 10 HEPES, pH 7.4] and incubated in the dark at room temperature with 2 μM fura 2-AM for 60 min. After fura loading, the VSMC were washed three times with HBSS and allowed to stabilize for 20 min. Cells were excited alternately with light of 340- and 380-nm wavelength from dual monochrometers of a Photon Technology International dual-excitation wavelength Deltascan (model RMD) interfaced with an inverted microscope (Olympus IX-70). After passing through a barrier filter (510 nm), fluorescence was detected by a photometer. Fluorescence signal intensity was acquired, stored, and processed by a Pentium II computer and Felix software (Photon Technology International). Each preparation was tested only once, to avoid possible receptor desensitization or tachyphylaxis.

RBF studies. Experiments were performed on 5- to 6-wk-old Sprague-Dawley rats derived from our Chapel Hill breeding colony by using standard methods for our laboratory (13). Anesthesia was induced by an intraperitoneal injection of pentobarbital sodium (65 mg/kg body wt), and the animals were placed on a servo-controlled heating table that maintained body temperature at 37°C. A tracheostomy was performed to facilitate free breathing. Carotid arterial pressure was monitored via an indwelling PE-50 catheter connected to a Statham P23 Db transducer and a Hewlett-Packard oscillographic recorder interfaced to an IBM-clone Pentium II computer. Maintenance infusions of isoosmotic albumin were made via a jugular vein. To measure RBF, a noncannulating electromagnetic flow probe (1.5-mm circumference, Carolina Medical) was placed around the left renal artery. A catheter inserted in a femoral artery was advanced into the abdominal aorta, and the tip was positioned at the origin of the renal artery for continuous infusion of heparinized isotonic saline (5 μl/min) and intrarenal injection of vasoactive agents. Before injection of EP agonists, the rate of saline infusion was increased to 120 μl/min; 60 s later a 10-μl bolus of the particular agent was injected into the catheter positioned in the renal artery. In some experiments, the EP1 blocker SC-19220 was infused into the artery at 120 μl/min for 2 min before injection of the PGE2 bolus. After completion of the surgical preparation, the animals were allowed to stabilize for 30 min before the measurements were started. The RBF and mean arterial pressure values were normalized and expressed as a percentage of baseline values, determined separately for each injection. Mean arterial pressure was constant during RBF responses to each injection. Plots of normalized RBF as a function of time were prepared by using the SigmaPlot software package (SPSS, Chicago, IL).

Statistics. Data are presented as means ± SE. Data sets concerning signal transduction were analyzed by analysis of variance followed by post hoc testing according to Student-Newman-Keuls. Maximum RBF responses were analyzed by Student’s t-test. P values <0.05 are considered statistically significant.

RESULTS

RT-PCR was used to evaluate gene expression of EP receptor subtypes in freshly isolated preglomerular arterioles and cultured preglomerular VSMC preparations. As is shown in Fig. 1A, signals indicating the presence of mRNA encoding EP1 and EP4 were detected in both fresh and cultured preparations by identity with the predicted product sizes of 415 and 318 bp, respectively. It should be noted that the primer used to identify EP1 mRNA does not differentiate between the two known splice variants of the EP1 receptor. PCR

![Image of PCR amplification](http://example.com/fig1a.png)

**Fig. 1.** RT-PCR amplification of fragments for the E-prostanoid (EP) receptor subtypes. PCR was performed by using RNA isolated from freshly isolated preglomerular microvessels (f), cultured preglomerular vascular smooth muscle cells (VSMC; c), and whole tissues (w). A: amplification fragments for both EP1 and EP4 are detectable. CYCLO, cyclophilin; cult, cultured. B: signals for EP2 and EP3 are undetectable in fresh and cultured renal VSMC, although fragments of the appropriate size are noted in tissues known to express the receptors, i.e., EP2 in lung and EP3 in kidney. C: although amplification fragments consistent with EP1-variant are found in both VSMC preparations, a band corresponding to EP1 mRNA is absent from cultured VSMC. For A–C, samples were electrophoresed on 1.5% agarose gels and visualized with ethidium bromide. Left: size markers. DNA quality was verified by amplifying each sample with cyclophilin primers. Data are representative of PCR reactions using 3 different sets of isolated microvessels and 3 separate batches of cultured VSMC.
products were directly sequenced and found to be identical to reported cDNA sequences for EP1 and EP4. Although we were able to amplify cyclophilin fragments from the same cDNA, expression of EP2 or EP3 message was undetectable in both fresh and cultured renal VSMC (Fig. 1B). It is unlikely that this finding of receptor absence is due to an error in primer design because subtype-specific primers produced fragments of the predicted size using cDNA samples from positive control clones as well as from tissues known to strongly express each subtype, lung for EP2 and kidney for EP3 (30, 36). The absence of bands in the RNA lanes indicates the elimination of genomic DNA contamination.

To assess the expression of the vasoconstrictor EP1 receptor in more detail, another set of PCR primers was designed to distinguish between the EP1 receptor and its variant, nonsignaling form. The EP1-variant consists of the first 951 bp of EP1, with an alternative sequence in place of the seventh transmembrane section containing a stop codon; the remaining sequence is common to both receptors. We designed the 5' primer to anneal within the first common region and the 3' primer to anneal to the terminal common sequence so that amplification of EP1-variant cDNA would produce a longer PCR fragment (730 bp) than native EP1 (360 bp). Figure 1C shows detection of amplification fragments indicative of the presence of the EP1-variant in both cultured and fresh preparations. Whereas EP1 expression was evident as a faint band in freshly isolated preglomerular microvessels, no signal was detected in cultured VSMC. A stronger band for EP1 was clearly observed in cDNA samples derived from whole kidney tissue. As before, the identity of these amplification fragments was confirmed by direct sequencing.

To examine the functional coupling of EP1 and EP4 receptors to Gi and or Gs proteins in freshly isolated preglomerular microvessels, cAMP levels were measured after stimulation of EP receptors by relatively subtype-specific agonists. Baseline production of cAMP by renal microvessels of Sprague-Dawley rats was 6.8 ± 2.5 pmol cAMP·50 mg protein⁻¹·ml⁻¹·15 min⁻¹, consistent with an earlier report in preglomerular VSMC isolated from Wistar-Kyoto and spontaneously hypertensive rat strains (33). All EP receptor agonists were tested at three concentrations; results for the highest concentration (10⁻⁵ M) are presented in Fig. 2. Sulprostone and 17-phenyl-PGE2, both putative EP1 and EP3 agonists, had no effect on cAMP. Because the EP1 receptor is thought to signal via inositol 1,4,5-triphosphate and [Ca²⁺], the lack of a change in cAMP after EP1 stimulation is not unexpected. Interpretation of results with EP3 stimulation is difficult because this receptor subtype can act via either activating or inhibiting adenylate cyclase. Butaprost, which is predicted to stimulate the EP2 receptor and presumably increase cAMP formation, had no effect on cAMP in our VSMC, suggesting a paucity of functional EP2 receptor. These data confirm our RT-PCR findings of the absence of EP2 mRNA transcripts. Addition of the nonselective EP2–4 stimulator misoprostol enhanced cAMP production by 4.3 ± 0.8 pmol cAMP·50 mg protein⁻¹·ml⁻¹·15 min⁻¹ (P < 0.05), a stimulatory effect consistent with our earlier observation of EP4 mRNA expression. The EP3 agonist M&B28767 had no effect on cAMP.

Fig. 2. Effect of EP-receptor agonists and antagonists (10⁻⁵ M) on cAMP production in freshly isolated preglomerular microvessels. Values are means ± SE for 3 preparations in each group. Misoprostol, an agonist of EP2–4 receptors, caused a significant increase when given alone (A; P < 0.05) or when coadministered with isoproterenol (B; P < 0.05). Other EP-receptor agonists were ineffective. C: both EP3 antagonist AH-23848 (P < 0.02) and EP1 antagonist AH-6809 (P < 0.03) decreased cAMP production in a dose-dependent manner.
In another set of experiments on the renal microvessels, cAMP production was initially stimulated by isoproterenol in an attempt to unmask any possible inhibition mediated by EP3 receptors. Isoproterenol elevated cAMP levels to 20.1 ± 1.8 pmol cAMP·50 mg protein⁻¹·ml⁻¹·15 min⁻¹ (P < 0.01). Coadministration of misoprostol further stimulated cAMP production by 8.5 ± 2.8 pmol cAMP·50 mg protein⁻¹·ml⁻¹·15 min⁻¹ (P < 0.05), whereas M&B28767 had no effect (Fig. 2B). These results are consistent with the notion that there is no EP3 receptor coupling to Gii proteins in our VSMC.

To evaluate the effect of available subtype-specific antagonists on PGE2-induced increases in cAMP, cells were pretreated with blockers for 5 min before PGE2 stimulation. As is shown in Fig. 2C, PGE2 alone produced 23.9 ± 1.2 pmol cAMP·50 mg protein⁻¹·ml⁻¹·15 min⁻¹ (P < 0.001). The EP4 antagonist AH-23848 dose dependently inhibited the ability of PGE2 to stimulate cAMP production. The highest AH-23848 dose tested reduced cAMP formation by 9.2 ± 1.1 pmol cAMP·50 mg protein⁻¹·ml⁻¹·15 min⁻¹ (P < 0.02). The EP1 antagonists were predicted to have no effect on PGE2-induced cAMP production due to presumed coupling to Gq proteins. Although this was clearly the case for SC-19220, AH-6809 (10⁻⁶ M) attenuated PGE2-induced increases in cAMP, cells were pretreated with blockers for 5 min before PGE2 stimulation, indicating low levels of misoprostol further stimulated cAMP production by 20.1 ± 5.6 pmol cAMP·50 mg protein⁻¹·ml⁻¹·15 min⁻¹ (P < 0.002). Neither sulprostone nor 17-phenyl-PGE2 altered baseline [Ca²⁺]i (32). Misoprostol did not alter baseline [Ca²⁺]i, but did dose dependently inhibit the [Ca²⁺]i response to ANG II (Fig. 3A). Maximal inhibition was reached at 10⁻⁵ M misoprostol, attenuating the peak [Ca²⁺]i response to ANG II from 245 ± 14.6 to 129 ± 8.8 nM (P < 0.002). Neither sulprostone nor 17-phenyl-PGE2 altered baseline [Ca²⁺]i, or the ANG II-induced [Ca²⁺]i response (Fig. 3B), as would be expected with the lack of expression of EP1 mRNA in cultured VSMC. Butaprost and M&B28767 were also without effect on calcium signaling (data not shown). These calcium data confirm the absence of EP2 and EP3 receptors in renal VSMC. Without EP2 and EP3 present, it is reasonable to conclude that misoprostol acts through the EP4 receptor to antagonize the actions of ANG II.

In other studies we assessed the effect of EP-receptor antagonists on ANG II-induced calcium responses. Figure 4A shows that the EP4-receptor antagonist AH-23848 dose dependently enhanced the peak level of calcium stimulation by ANG II. At the highest concentration of the EP4-receptor antagonist (10⁻⁵ M), the response nearly doubled to 462 ± 30.5 nM (P < 0.002). AH-23848 did not alter baseline [Ca²⁺]i when administered in the absence of ANG II, indicating low levels of EP4 receptor stimulation during basal conditions. Figure 4B reveals that the EP1 antagonists AH-6809 and SC-19220 affected neither baseline [Ca²⁺]i nor ANG II-induced calcium signaling, findings that are consistent with the lack of PCR evidence for EP1 in cultured preglomerular VSMC.

To verify the functional expression of EP receptor subtypes in renal resistance arterioles in vivo, we examined vascular reactivity by measuring changes in RBF after administration of PGE2 and subtype-specific agents into the renal artery. Basal values for RBF and mean arterial pressure averaged 5.8 ± 0.6 ml/min and 112 ± 5 mmHg (n = 15), respectively. A bolus of the EP1-receptor agonist sulprostone (200 ng) caused transient renal vasoconstriction, reducing RBF to 91.9 ± 1.2% of control flow (P < 0.001) (Fig. 5A), without affecting systemic arterial pressure. In contrast, misoprostol (200 ng) elicited a longer lasting increase in RBF to 106 ± 0.8% of control flow (P < 0.001), indicating renal vasodilation in the absence of a change in arterial pressure (Fig. 5B). To determine whether the EP3 subtype expressed in fresh renal preglomerular VSMC is functional in vivo, rats were challenged with...
PGE$_2$. Bolus injection of PGE$_2$ (200 ng) into the renal artery produced a biphasic response, characterized by immediate, short-lived constriction followed by a more sustained phase of dilation (Fig. 5). During the immediate transient constriction, RBF fell to 85.3 ± 4.2% of control (P, 0.02), followed by a longer lasting dilatory phase in which RBF rose to a peak of 110.8 ± 0.9% of baseline (P, 0.001). The constrictor period of ~15 s, contrasted with the prolonged dilatory phase, returned to control levels after 5 min. It is noteworthy that the initial constrictor response was completely abolished by 2-min pretreatment with the EP$_1$ blocker SC-19220. Selectivity was indicated by the lack of change in the sustained, elevated phase during SC-19220 administration.

DISCUSSION

We present new evidence that preglomerular resistance vessels of the rat kidney express two of the four known EP receptors, with EP$_1$ causing transient vasoconstriction and EP$_4$ eliciting long-lasting vasodilation. As PGE$_2$ produces net vasodilation, EP$_4$ is the functionally predominant receptor. Detection of the EP$_4$ receptor provides a mechanism for the long-standing observation that prostaglandins can effectively buffer the actions of vasoconstrictors in the renal microvasculature and thereby protect kidney function (11, 25). EP$_1$ receptor expression is evident pharmacologically at the whole kidney level in vivo, and message is localized by RT-PCR to the preglomerular resistance vessels. Also present in freshly isolated microvessels is mRNA encoding a nonsignaling isoform, termed the EP$_1$-variant. Interestingly, EP$_1$ receptors are absent in cell culture, whereas the EP$_1$-variant and EP$_4$ receptors are retained. These observations provide a cellular...
The presence and functional expression of EP4 in rat arterioles elucidate the mechanisms underlying the well-known vasodilatory actions of PGE2. In the renal microcirculation, it has been clear for many years that PGE2 can oppose constriction in preglomerular vessels (6, 11, 22, 25). The activation of EP4 by PGE2 is crucial to maintain RBF when vasoconstrictor systems are active as, for example, during extracellular fluid volume contraction and in disease states. In regard to experimental models, previous investigations have established that an imbalance between vasodilatory and vasoconstrictor systems may contribute to the development of hypertension. In young spontaneously hypertensive rats, the dysfunction appears to arise in the interaction between a vasodilatory prostaglandin receptor and the Gs protein that activates adenylate cyclase (32). On the basis of the present results, it is reasonable to predict that this defect lies in the coupling of the EP4 receptor to Gs proteins and the cAMP pathway.

The expression of vasoconstrictor EP1 receptors was unexpected in view of the noted predominant vasodilatory actions of PGE2. Nevertheless, message for this subtype has been reported in isolated glomeruli (23) and cultured mesangial cells (21). Our RBF studies establish functional expression of the vasoconstrictor EP1 subtype in the microcirculation in vivo. The immediate transient decrease in RBF caused by PGE2 injection into the renal artery is fully abolished by the EP1 antagonist SC-19220 while having little, if any, effect on the more pronounced, sustained vasodilation. Moreover, the EP1 agonist sulprostone elicits renal vasoconstriction in our Sprague-Dawley rats. The fact that sulprostone does not alter cAMP in our VSMC preparation supports the notion that its actions are mediated by EP1, rather than EP3, in vivo. Interestingly, previous studies of the effect of PGE2 on RBF in our laboratory demonstrated a vasodilator effect only, suggesting there may be strain differences in EP receptor subtype expression (12).

Of particular interest are the changes in EP4 expression in renal VSMC maintained in cell culture. Sulprostone as well as the EP1 antagonists AH-6809 and SC-19220 had no effect on the putative EP1 signaling pathway, cysotolic calcium, in cultured VSMC. This loss of expression during primary cell culture is most clearly evidenced by the detection of EP1 message in freshly isolated cells and its absence in cultured cells. A similar loss of EP receptors in culture has been reported in rabbit cortical collecting tubule cells, evidenced by a decrease in PGE2 binding and a loss of inhibition of cAMP formation (34). The altered distribution of EP receptor expression in cultured VSMC may impact future studies of prostaglandin action. Although global PGE2 function cannot be assessed in cultured preparations, the cultured VSMC could facilitate the study of EP4 actions without confounding interactions with EP1 signaling.

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wild-type control animals, systemic administration of PGE₂ lowered arterial pressure less in mice in which the coding sequence common to EP₁ and EP₄-variant receptors was disrupted by gene targeting. Thus the enhanced depressor response in wild-type mice presumably reflects an unexpected contribution of EP₁ receptors to apparent vasodilation, although cardiac effects cannot be excluded. Our RBF data clearly demonstrate that the EP₁ receptor mediates vasoconstriction in the renal vasculature of Sprague-Dawley rats. This divergence in EP₁ function may be explained by heterogeneous effects of EP receptor activation in various vascular beds or species differences in receptor signaling. Nevertheless, it is important to appreciate that renal vasoconstriction mediated by EP₁ is transient and plays a weaker role compared with that of the prolonged vasodilation mediated by EP₄. Perhaps the expression of this vasoconstrictor receptor provides a means of further modulating the prostaglandin effect in the microcirculation. It is also possible that EP₁ is expressed preferentially in a subpopulation of minority nephrons with EP₄ as the predominant receptor in the more numerous population of cortical nephrons. Such a model is consistent with a previous study demonstrating solely vasoconstrictive effects of PGE₂ in the juxtamedullary nephron preparation (20).

The only other study to differentiate between EP₁ and EP₄-variant expression is the original work in which the EP₁ receptor variant was cloned (31). Our results document for the first time mRNA expression for the EP₁-variant in both cultured and freshly isolated preglomerular preparations. The EP₁-variant receptor displays the same ligand binding specificity as EP₁ but lacks the COOH-terminal signaling domain (31). Thus the function, or lack thereof, of this subtype is difficult to establish because the portion of the receptor that couples to the G protein is truncated, and the receptor does not appear to generate signals along traditional transduction pathways. One can speculate that the expression of EP₁-variant protein at the cell surface is regulated to finely tune the response to PGE₂. This variant receptor may serve as a type of clearance receptor, removing excess PGE₂ from the circulation and guarding against the sudden onset of dangerously low blood pressure. It may bind PGE₂ and release it slowly to VSMC in a manner similar to that postulated for the similarly truncated form of the atrial natriuretic peptide receptor (24). Also, the possibility that the EP₁-variant couples to an alternative signaling pathway, such as growth regulation or metabolism, cannot be ruled out.

Species comparisons of EP renal vascular expression are complicated by conflicting results. Although ribonuclease protection assays detect primarily transcripts for EP₄ with lower levels of EP₁ and EP₃ in freshly isolated rat glomerular tissue (23), cultured mesangial cells are reported to express EP₁ and EP₄ (21). In mice, only EP₂ transcripts are detected in glomeruli (35). Similarly, in situ hybridization experiments using human tissue support solely EP₄ expression (10), whereas immunohistochemistry studies detect both EP₂ and EP₄ in human glomeruli (28). The latter study examined expression in the preglomerular vasculature, and reactivity for EP₁₋₃, but not EP₄, was reported (28). It is possible that the differences in arteriolar expression may also reflect species variation. Nevertheless, it should be emphasized that our findings concerning EP receptors in rat preglomerular VSMC are based on multiple techniques examining gene expression, cellular signaling ability, and in vivo responses, in contrast to a human study that is confined to antibody recognition of protein.

Our comprehensive findings in the preglomerular vasculature extend the several studies that have examined EP receptor expression in cultured rat glomerular mesangial cells to physiological control of renal vascular resistance and glomerular hemodynamics. Measurements of changes in cross-sectional area suggested both constrictor and dilatory actions of PGE₂ in cultured mesangial cells (26). Furthermore, PGE₂ has been shown to evoke an increase in cytosolic calcium that can be blocked by EP₁ antagonists. The receptor subtype responsible for the increase in cAMP was not identified. In addition, mRNA message for both EP₁ and EP₄ was detected by Northern blotting in cultured rat mesangial cells (21). A comparison of results provide evidence for phenotypic differences between cultured cells derived from preglomerular resistance arterioles and glomeruli on the basis of EP₁ receptor expression. It is not clear whether mesangial cells express the EP₁-variant because the riboprobe used was nonselective.

Of particular interest is the potential therapeutic utility of EP subtype-specific drugs. Nonsteroidal anti-inflammatory drugs, which inhibit the cyclooxygenase enzyme that produces PGE₂, are presently among the most widely prescribed drugs. In volume-compromised conditions, administration of nonsteroidal anti-inflammatory drugs can severely reduce RBF and lead to reversible acute renal failure (39). These undesirable side effects arise from inhibiting renal PGE₂ production and actions, which lead to tubular as well as vascular disturbances. With the high probability that EP₄ tranduces the protective vasodilator signal in the renal microcirculation, it is tempting to speculate about the development of EP receptor-specific agents that would be more selective for desired vascular effects and free of the undesirable side effects of present medications. Therapeutic drugs specific to EP receptors may allow better treatment of conditions of extracellular fluid volume contraction and hyperdynamic vasoconstrictor status in pathophysiological conditions.

In summary, the present study provides important new information about gene expression and functional characterization of EP₁ and EP₄ receptors localized to preglomerular resistance arterioles of Sprague-Dawley rats. In contrast, message for EP₂ and EP₃ was absent and no functional effect of these receptor subtypes was observed. Preglomerular VSMC express an EP₁-variant receptor that does not appear to possess any cAMP or cytosolic calcium signaling capable of affecting RBF.
In vivo RBF studies indicate that the EP<sub>4</sub> receptor is the primary receptor mediating sustained renal vasodilation produced by PGE<sub>2</sub>, whereas the EP<sub>1</sub> receptor can produce transient vasoconstriction. We conclude that the EP<sub>4</sub> receptor is responsible for PGE<sub>2</sub>-induced vasodilation in the rat kidney, signaling through G<sub>s</sub> proteins and the cAMP pathway to reduce [Ca<sup>2+</sup>]i when stimulated by a vasoconstrictor such as ANG II.

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