Biphasic Actions of Prostaglandin E₂ on the Renal Afferent Arteriole
Role of EP₃ and EP₄ Receptors

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Abstract—Prostaglandin (PG) E₂ is an important modulator of the actions of angiotensin (Ang) II. In the present study, we investigated the renal microvascular actions of PGE₂ and the EP receptor subtypes involved. Ibuprofen potentiated Ang II–induced vasoconstriction in in vitro perfused normal rat kidneys and augmented afferent arteriolar, but not efferent arteriolar, responses in the hydronephrotic rat kidney model. This preglomerular effect of endogenous prostanoids was mimicked by exogenous PGE₂, which reversed Ang II–induced afferent arteriolar vasoconstriction at concentrations of 0.1 to 10 nmol/L without affecting the efferent arteriole. The PGE₂-induced vasodilation was potentiated by the phosphodiesterase inhibitor Ro 20-1724 and was mimicked by 11-deoxy-PGE₁ (0.01 to 1 nmol/L). Butaprost, which acts preferentially at EP₂ receptors, was relatively ineffective. Whereas 0.1 to 10 nmol/L PGE₂ elicited vasodilation, higher concentrations (1 to 10 μmol/L) restored Ang II–induced afferent arteriolar vasoconstriction. This response was blocked by pertussis toxin (200 μg/mL) and was mimicked by the EP₁/EP₃ agonist sulprostone (1 to 300 nmol/L). Reverse transcription–polymerase chain reaction of individually isolated afferent arterioles revealed the presence of message for EP₄ and all 3 EP₃ splice variants (α, β, and γ) but not EP₁ or EP₂. Our findings thus indicate that PGE₂ elicits both vasodilatory and vasoconstrictor actions on the afferent arteriole. The vasodilation is mediated by EP₄ receptors coupled to cAMP, presumably via Gₛ. The vasoconstriction is mediated by an EP₃ receptor coupled to Gₛᵢ and appears to reflect a functional antagonism of the EP₄-induced vasodilation. (Circ Res. 2000;86:663-670.)

Key Words: receptors ▪ microcirculation ▪ arterioles ▪ cyclooxygenase ▪ angiotensin II

Prostaglandin (PG) E₂ is the major prostanoid produced by the kidney and plays an important role in modulating the renal microvascular response to vasoconstrictors.¹ During conditions associated with low renal perfusion pressure, elevated renin activity, and increased renal sympathetic traffic, normal renal function may depend on the modulatory actions of PGE₂ on angiotensin (Ang) II– and catecholamine-induced renal vasoconstriction. In clinical settings such as congestive heart failure, interference with this compensatory mechanism by the administration of nonsteroidal anti-inflammatory agents can lead to a deterioration of renal function and renal insufficiency.² It has now been disclosed that the diverse biological actions of PGE₂ are mediated by specific members of a group of G protein–linked (EP) receptors (reviewed in Reference 3). Thus, future therapies may allow a more selective intervention based on the development of selective receptor antagonists capable of blocking undesirable effects of the prostanoids while preserving their renal protective actions. This approach will require information on the renal microvascular actions of PGE₂ and the identification of the specific EP receptor subtypes involved.

Although PGE₂ is recognized to play a critical role in modulating renal vasoconstriction, the actions of PGE₂ on the renal arterioles have not been thoroughly investigated. Only 2 studies to date have directly examined the renal microvascular actions of PGE₂. In a study of isolated rabbit arterioles, Edwards⁴ reported that PGE₂ elicited afferent arteriolar vasodilation but had no effect on the efferent arteriole. No constrictor responses to PGE₂ were observed in this study. In contrast, Inscho et al.,⁵ using the blood-perfused juxtamedullary nephron preparation, found 1.0 μmol/L PGE₂ to evoke afferent arteriolar vasoconstriction in the rat. Only 1 concentration was used, and the effects of PGE₂ on the efferent arteriole were not examined. Neither study examined the EP receptor subtypes or signaling pathways involved.

At least 4 distinct genes encoding for 4 separate subtypes of PGE₂ receptors, EP₁ to EP₄ (reviewed in Reference 3), have been identified. The mRNA for all 4 EP receptor subtypes has been reported to be expressed in the kidney.⁶⁻⁹ EP₂ and EP₄ receptors are generally associated with smooth muscle relaxation, whereas EP₁ and EP₃ receptors are reported to elicit smooth muscle contractile responses. However, although the receptor subtypes involved in the tubular epithelial actions of PGE₂ have been partially characterized (reviewed in Reference 10), we know little of the EP receptor subtypes and
signaling pathways involved in the renal microvascular actions of PGE\(_2\). In the present study, we used the in vitro perfused normal rat kidney and the in vitro perfused hydronephrotic rat kidney models to investigate the actions of PGE\(_2\) on the afferent and efferent arterioles. We used reverse transcription–polymerase chain reaction (RT-PCR) assays of microdissected vessels to determine EP receptor subtype expression in the renal afferent arteriole. Our findings indicate that PGE\(_2\) exerts a selective effect on the afferent arteriole, eliciting both vasodilation and vasoconstriction of this vessel. The vasodilation appears to be mediated by the EP\(_4\) receptor coupled to a cAMP-dependent mechanism. The vasoconstrictor actions of PGE\(_2\) are mediated by an EP\(_3\) receptor coupled to a G\(_\text{ai}\) and appear to involve a functional antagonism of the EP\(_4\)-induced vasodilation.

**Materials and Methods**

In Vitro Perfused Hydronephrotic Kidney

Male Sprague-Dawley rats were used in all experiments. The in vitro hydronephrotic rat kidney\(^1\)\(^1\) was used to examine the afferent and efferent arteriolar actions of PGE\(_2\). Unilateral hydronephrosis was induced by ligating the left ureter. Kidneys were harvested after 6 weeks, when tubular atrophy allows a direct visualization of the renal microvasculature. The renal artery was cannulated, and the kidney was excised with continuous perfusion. Kidneys were perfused at a renal arterial pressure of 80 mm Hg, with a modified Dulbecco’s medium containing 30 mmol/L bicarbonate, 5 mmol/L glucose, and 5 mmol/L HEPES and equilibrated with 95% air/5% CO\(_2\) (37°C, pH 7.4). Diameters were measured by online image processing. Afferent diameters were measured between the midpoint and origin. Efferent arteriolar diameters were measured within 50 \(\mu\)m of the glomerulus.

Initial studies were performed to determine the effects of basal prostaglandins. In all subsequent studies, kidneys were pretreated with 10 \(\mu\)mol/L ibuprofen to eliminate endogenous prostanoids. PGE\(_2\) was obtained from Calbiochem and prepared fresh daily. Ro 20-1724 was obtained from Research Biochemicals International and was administered at 0.5 \(\mu\)mol/L. Sulprostone and 11-deoxy-PGE\(_2\) were obtained from Cayman Chemical Co. Butaprost was provided by Dr Kluender of Bayer Pharmaceutical Division. SC-51322 was obtained from BIOMOL Research Laboratories.

In Vitro Perfused Normal Kidney

For the normal kidney studies, rats weighing from 220 to 275 g were anesthetized with methoxyflurane. The left renal artery was cannulated in situ, and the kidney was excised with continuous perfusion. Kidneys were perfused in vitro at 80 mm Hg as described above. Perfusate flow was measured with a transonic flowmeter (model T106, Transonic Systems, Inc).

**EP mRNA Expression in Isolated Afferent Arterioles**

Normal kidneys were perfused with 2% agarose (37°C in DMEM) and chilled (4°C). Cortical slices (400 \(\mu\)m) were prepared and treated with collagenase IV, hyaluronidase IV, dispase II, and DNase I. Six to 8 arterioles were individually isolated and washed 3 times. RT-PCR was run on the final wash to test for contamination. Arteriolar total RNA was extracted, and cDNA was synthesized with random primers. To eliminate genomic contamination, a separate sample was treated identically, but without reverse transcriptase (Figure 8A and 8B). Primers for RT-PCR assays were designed from the reported EP receptor sequences\(^{12–17}\) (Table 1 online, see http://www.circresaha.org). We assayed for a sequence common to all EP\(_1\) receptors (EP\(_{\text{iso}}\)) and sequences specific for EP\(_1\), EP\(_2\), EP\(_3\), and EP\(_4\) splice variant. A whole-kidney homogenate was used as a positive control, and primers for GAPDH\(^{18}\) were used for an internal control. After 35 cycles, PCR products were separated by electrophoresis (2% agarose gel) and stained with ethidium bromide. The identity of the products obtained with each set of primers was confirmed by DNA sequencing. When negative results were obtained, fresh polymerase was added after 35 cycles, and the PCR was continued to 70 cycles.

**Analysis**

Data are expressed as the mean±SEM. Differences were evaluated by ANOVA and Student’s \(t\) test (paired or unpaired). Values of \(P<0.05\) were considered significant. For multiple comparisons, the Bonferroni correction was applied, and values of \(P<0.05/n\) were considered significant.

An expanded Materials and Methods section is available online at http://www.circresaha.org.

**Results**

Initial studies were conducted to determine the influence of endogenous prostanoids on Ang II responses of the in vitro perfused hydronephrotic kidney preparation and of in vitro perfused normal rat kidneys. The effects of 10 \(\mu\)mol/L ibuprofen on the afferent and efferent arteriolar actions of Ang II are presented in Figure 1A. Basal diameters were not affected by ibuprofen in either the afferent (15.6±1.8 versus 15.4±1.6 \(\mu\)m, \(n=8, P=0.84\)) or efferent (11.2±1.2 versus 11.7±1.2 \(\mu\)m, \(n=8, P=0.68\)) arteriole. In the afferent arte-
perfusate flows were 13.4 ± 0.0036. As presented in Figure 1B, ibuprofen significantly altered Ang II–induced vasoconstriction (0.1 nmol/L, *P < 0.003) in controls and at 0.03 nmol/L (11.4 ± 1.4 μm, *P = 0.0016) in the presence of ibuprofen. As shown in Figure 1A, ibuprofen pretreatment resulted in significant potentiation of the afferent arteriolar response at Ang II concentrations of 0.01 to 0.1 nmol/L (*P < 0.05). In contrast, the efferent arteriolar response to Ang II was not significantly altered by ibuprofen, and 0.03 nmol/L Ang II elicited significant efferent arteriolar vasoconstriction in both settings.

Ibuprofen also potentiated Ang II–induced vasoconstriction in normal perfused rat kidneys (Figure 1B). In the control group, basal perfusate flows were 14.6 ± 0.9 mL · min⁻¹ · g kidney wt⁻¹ (n = 5), and Ang II elicited significant vasoconstriction at 0.1 nmol/L (9.5 ± 1.0 mL · min⁻¹ · g⁻¹, *P < 0.0001). In kidneys pretreated with 10 μmol/L ibuprofen, basal perfusate flows were 13.4 ± 0.6 mL · min⁻¹ · g⁻¹ (*P = 0.26 versus control, n = 5) and Ang II elicited significant vasoconstriction at 0.03 nmol/L (11.8 ± 0.5 mL · min⁻¹ · g⁻¹, *P = 0.0036). As presented in Figure 1B, ibuprofen significantly potentiated the vasoconstrictor response to Ang II at concentrations of 0.03 and 0.1 nmol/L (*P < 0.05). In concert, the findings presented in Figures 1A and 1B illustrate that the threshold for Ang II–mediated vasoconstriction occurs over similar concentrations in the in vitro perfused hydronephrotic kidney and normal kidney preparation and that cyclooxygenase inhibition potentiates reactivity to Ang II in both models.

Because the major prostaglandin produced by the kidney is PGE₂, we examined the effects of exogenous PGE₂ on the afferent and efferent arteriolar response to Ang II. In these studies and those that follow, kidneys were pretreated with 10 μmol/L ibuprofen to eliminate endogenous prostanoid formation. As shown in Figure 2, 0.1 nmol/L Ang II reduced afferent arteriolar diameter by 43 ± 7% (n = 8) and efferent arteriolar diameter by 23 ± 5% (n = 7, *P < 0.001). PGE₂ exerted a biphasic action on the afferent arteriole. Over concentrations ranging from 1 to 10 nmol/L, PGE₂ elicited afferent arteriolar vasodilation, completely reversing the Ang II–induced vasoconstriction at a concentration of 10 nmol/L (14.2 ± 0.6, 8.2 ± 1.2, * and 13.4 ± 0.7 μm for control, Ang II, and Ang II plus 10 nmol/L PGE₂, respectively; *P < 0.05 versus control), increasing the PGE₂ concentration to 1.0 μmol/L fully restored the vasoconstriction (7.0 ± 1.4 μm, *P > 0.05 versus Ang II alone). In the absence of Ang II (Figure 2, top), PGE₂ also elicited a modest vasodilation and constriction at concentrations of 10 nmol/L and 1 μmol/L (P < 0.05). However, the constrictor actions of PGE₂ (1.0 μmol/L) were far greater in the presence of Ang II (51 ± 8% decrease in diameter) than in its absence (11 ± 3% decrease). Finally, in contrast to the afferent arteriole, the efferent arteriolar actions of Ang II were not significantly altered by PGE₂ over concentrations ranging from 1.0 nmol/L to 1.0 μmol/L (Figure 2, bottom).

To investigate the signaling pathway involved in the vasodilation, we determined the effects of the specific type IV phosphodiesterase inhibitor Ro 20-1724. A concentration of 0.5 μmol/L Ro 20-1724 was selected for these studies because we found that this concentration does not produce vasodilation by itself but rather potentiates the vasodilatory actions of adenosine (not shown). Two groups of hydronephrotic kidneys (controls and Ro 20-1724–treated, n = 5 for each) were preconstricted with 0.1 nmol/L Ang II, and the afferent arteriolar vasodilatory actions of PGE₂ were assessed. The inhibition of the Ang II–induced vasoconstriction was calculated, and these data are summarized in Figure 3. Ro 20-1724 markedly potentiated the actions of PGE₂, shifting the ED₅₀ by ~1 log unit. These findings are consistent with the currently held view that the vasodilatory actions of PGE₂ are mediated by cAMP.

Both EP₁ and EP₄ have been shown to be coupled to Gₛ and are thought to produce vasodilation by stimulating the
production of cAMP. To characterize the receptor involved in the afferent arteriolar vasodilation, we compared the potency of 11-deoxy-PGE, and butaprost. These 2 agonists have similar affinities for the EP2 receptor but markedly different affinities for the EP4 receptor. Figure 4 summarizes the results of studies comparing the actions of these 2 agonists on afferent arterioles preconstricted with 0.1 nmol/L Ang II (n=5 for both groups). As depicted, 11-deoxy-PGE1 fully reversed Ang II–induced afferent arteriolar vasoconstriction (ED50≈0.1 nmol/L). In contrast, butaprost was a relatively weak vasodilator in this preparation. These results are consistent with an EP4–mediated vasodilation.

The biphasic response of the afferent arteriole to PGE2 indicates actions at multiple receptor subtypes, linked to both vasodilator and vasoconstrictor mechanisms. To characterize the constrictor response, we examined the effects of pretreatment with pertussis toxin (PTX). In these studies, kidneys were pretreated with 200 μg/mL PTX for 1 hour with a recirculating circuit. Control kidneys were treated in an identical manner with vehicle alone. The kidneys were then returned to the single-pass perfusion system and challenged with Ang II and PGE2. As shown in Figure 5, PTX had no effect on the magnitude of the Ang II–induced vasoconstriction and did not alter the vasodilatory actions of 10 nmol/L PGE2. However, PTX treatment completely abolished the vasoconstrictor actions of 1.0 μmol/L PGE2.

Of the known PGE2 receptors, the EP3 receptor subtype has been shown to be coupled to the PTX-sensitive G protein Gαi. We next examined the effects of the EP3 receptor agonist sulprostone. As shown in Figure 6A, sulprostone did not elicit afferent arteriolar vasodilation in kidneys preconstricted with Ang II and, in this setting, had no vasoconstrictor activity. However, as described above (Figure 2), the vasoconstrictor actions of PGE2 were much more evident when administered to preconstricted vessels that were then dilated with low concentrations of PGE2. We therefore designed studies to mimic this condition, first inducing vasoconstriction with Ang II, then eliciting full vasodilation with 10 nmol/L PGE2, and then administering sulprostone (Figure 6B). Under these conditions, sulprostone caused a dose-dependent vasoconstriction, eliciting threshold contractions at concentrations of 1.0 to 10 nmol/L. Because sulprostone can activate both EP1 and EP3 receptors, we examined the effects of SC-51322, an EP1-selective antagonist (pA2=8.1), on this response. As shown in Figure 1 online (see http://www.cirresaha.org), SC-51322 at concentrations of 3 and 10 μmol/L had no effect on the sulprostone-induced vasoconstriction, suggesting that this response is mediated by EP3 receptors.

The results of the above studies suggest that an EP3 receptor coupled to a PTX-sensitive G protein mediates the afferent arteriolar vasoconstrictor effects of PGE2 in the hydronephrotic kidney model. To determine whether a similar response occurs in the nonhydronephrotic kidney, parallel studies were performed using isolated perfused normal kidneys. Kidneys were treated with 10 μmol/L ibuprofen and perfused at a constant arterial pressure of 80 mm Hg.
constriction was established with 0.1 nmol/L Ang II, and then 10 nmol/L PGE₂ was administered. As depicted in Figure 7, PGE₂ partially reversed the Ang II–induced vasoconstriction, consistent with a selective afferent arteriolar vasodilation. In this setting, sulprostone elicited a similar vasoconstrictor response.

Finally, to determine the expression of EP receptors in the renal afferent arteriole, RT-PCR was used to assay EP receptor mRNA in individually isolated afferent arterioles. As depicted in Figure 8A, the presence of afferent arteriolar message for EP₁ (EP₁α,β,γ) and EP₄ receptors, but not for EP₁ or EP₂ receptors, could be demonstrated with this approach. To further identify the EP₁ receptors present, individual primers for mRNA regions specific for the α, β, and γ splice variants were used. As shown in Figure 8B, we found the mRNA for all 3 splice variants to be expressed in the normal afferent arteriole.

Discussion

The diverse actions of PGE₂ on the kidney are mediated by a distinct group of G protein–coupled receptors. Four genes encoding for differing PGE₂ receptors have been cloned (EP₁ to EP₄), and message for all 4 receptor subtypes is found in the kidney. Previous studies have focused on the EP receptors mediating the renal tubular actions of PGE₂, but no information is currently available on the renal microvascular EP receptors. The present study indicates that the afferent arteriole of the rat expresses message for the EP₁ receptor and for 3 known splice variants of the EP₁ receptor (α, β, and γ). We found PGE₂ to elicit vasodilation at low concentrations (ED₅₀ <3 nmol/L) by activating EP₁ receptors coupled to cAMP. This vasodilation is reversed at higher concentrations (>100 nmol/L). This vasconstrictor response is mediated by an EP₁ receptor coupled to Gₐ and reflects a functional antagonism of the EP₄-mediated vasodilation.

The present study used the hydronephrotic rat kidney model to assess microvascular responses. Although this model is a widely used method of studying the renal microvasculature, it was important to characterize the role of endogenous prostanoids in this preparation. During the onset of hydronephrosis, infiltrating macrophages increase renal thromboxane production, eliciting vasoconstriction. For microvascular studies, a long-term preparation (6 to 8 weeks’ duration) is used. At this stage, tubular atrophy is complete, renal thromboxane production returns to normal levels, and SQ29548 has no effect on basal tone, indicating a lack of thromboxane-dependent vasoconstriction. The reactivity of our in vitro model to Ang II was similar to that of the normal kidney, because threshold vasoconstrictor responses were observed at concentrations of 0.03 to 0.1 nmol/L in both preparations. Moreover, the reactivity to Ang II was similarly potentiated by cyclooxygenase inhibition, suggesting a predominant influence of endogenous vasodilatory prostanoids in both models (Figure 1A and 1B).
dogenous prostanoids in our model. These findings are consistent with previous observations using in vivo preparations. For example, Heller and Horacek\(^2\) and Olsen et al\(^3\) found that cyclooxygenase inhibition potentiates the ability of Ang II to reduce glomerular filtration rate in the dog. These authors suggested a protective role of vasodilatory prostanoids in suppressing Ang II-mediated afferent arteriolar vasoconstriction. Our results are in agreement with this interpretation and suggest that the prostanooids involved in this action is PGE\(_2\), the major prostanooid produced by the normal kidney\(^1\) and by cortical interstitial cells of the hydronephrotic kidney.\(^26\) Like the endogenous prostanooids, exogenous PGE\(_2\) altered the afferent arteriolar effects of Ang II but had no effect on the efferent arteriole. This selective afferent arteriolar effect of PGE\(_2\) observed in our rat kidney model agrees with previous findings by Edwards,\(^4\) who used isolated afferent and efferent arterioles from the rabbit. Edwards reported PGE\(_2\) to block the afferent arteriolar response to norpinephrine but to have no effect on the efferent arteriolar actions of either norpinephrine or Ang II. Ang II does not constrict the afferent arteriole in Edwards’ preparation.\(^31\)

At concentrations >100 nmol/L, we found PGE\(_2\) to promote afferent arteriolar vasconstriction. A number of previous studies have documented both vasodilator and vasoconstrictor actions of PGE\(_2\) (reviewed in Reference 1). Edwards did not observe PGE\(_2\)-induced vasoconstriction in isolated rabbit arterioles.\(^4\) However, in a study using the blood-perfused juxtaglomerular nephron preparation of the rat, Inscho et al\(^6\) found PGE\(_2\) to elicit afferent arteriolar vasoconstriction. In the Inscho study, only a single concentration of PGE\(_2\) (1.0 \(\mu\)mol/L) was used, and efferent arteriolar responses were not reported. Our observation that 1.0 \(\mu\)mol/L PGE\(_2\) elicited afferent arteriolar vasoconstriction in the hydronephrotic kidney model is consistent with these findings. The fact that PGE\(_2\) elicits both constrictor and dilator responses clearly indicates an involvement of multiple EP receptor subtypes.

EP\(_1\) and EP\(_2\) receptors are both linked to vasodilatation (reviewed in Reference 3). Our studies indicate that in the afferent arteriole, PGE\(_2\) elicits vasodilatation by activating EP\(_2\) receptors. In this vessel, we found 11-deoxy-PGE\(_1\) to be a potent vasodilator, whereas butaprost was relatively ineffective (Figure 4). Butaprost and 11-deoxy-PGE\(_1\) are both EP\(_2\) agonists and exhibit similar potencies at this receptor subtype.\(^3,13,19\) However, 11-deoxy-PGE\(_1\) is also a potent EP\(_1\) agonist, whereas butaprost has very weak activity at this receptor.\(^3,13,19\) Thus, the rank order of potencies we observed (11-deoxy-PGE\(_1\) \(\gg\) butaprost) suggests that the vasodilation involves the EP\(_1\) rather than the EP\(_2\) receptor. This interpretation is consistent with the results of our RT-PCR studies, in that we found that message for EP\(_1\) but not EP\(_2\) was expressed in the afferent arteriole. We could not demonstrate the presence of EP\(_1\) mRNA even under extreme conditions (70 cycles). Previous studies have reported low expression levels of EP\(_3\) mRNA in the kidney and an absence of this signal in the renal cortex.\(^6\) We also found low levels of EP\(_3\) mRNA in whole-kidney homogenates (Figure 8A). In contrast, EP\(_2\) receptor mRNA is highly expressed in the kidney and has been localized to the glomerulus.\(^6,7\) Both EP\(_2\) and EP\(_4\) receptors have been shown to be coupled to G\(_{\alpha_i}\) and stimulation of adenyl cyclase (reviewed in Reference 3). The phosphodiesterase inhibitor Ro 20-1724 potentiated the vasodilatory response to PGE\(_2\) in our study, a finding in agreement with previous observations suggesting that the renal microvascular actions of PGE\(_2\) are mediated by an elevation in cAMP.\(^1,32\) Our observations are thus consistent with a signaling pathway involving PGE\(_2\) stimulation of EP\(_4\) receptors, leading to the activation of G\(_{\alpha_i}\), adenyl cyclase, and vasodilation via cAMP-dependent mechanisms.

Our studies suggest that the vasoconstrictor actions of PGE\(_2\) involve an EP\(_3\) receptor. Sulprostone caused vasoconstriction at concentrations of 10 to 100 nmol/L, whereas constrictor responses to PGE\(_2\) occurred only at much higher concentrations (0.3 to 1.0 \(\mu\)mol/L). Sulprostone acts on both EP\(_1\) and EP\(_2\) receptors but is less potent than PGE\(_2\) on EP\(_1\) receptors and more potent on EP\(_3\) receptors.\(^3,13,20\) The relative potency of sulprostone versus PGE\(_2\) is thus consistent with an EP\(_3\) response. This interpretation is supported by our observation that SC53122, an EP\(_3\)-selective antagonist, did not alter the vasocostrictor response to sulprostone. The RT-PCR studies are also consistent with an involvement of EP\(_1\) rather than EP\(_3\) receptors, because we found that the afferent arteriole expresses message for all 3 splice variants of the rat EP\(_1\) receptor, but we could not demonstrate message for EP\(_3\) in this vessel. Finally, the contractile response to PGE\(_2\) was prevented by PTX, indicating a coupling to G\(_{\alpha_i}\) or G\(_{\alpha_q}\) EP\(_3\)-mediated responses are insensitive to PTX and generally linked to G\(_{\alpha_q}\) whereas G\(_{\alpha_i}\) coupling is a common characteristic of EP\(_3\) receptors.\(^3,5,3,53\) In concert, these observations provide compelling evidence that the EP\(_3\) receptor mediates PGE\(_2\)-induced afferent arteriolar vasodilation.

Thus, our functional studies indicate that in the rat, the renal microvascular actions of PGE\(_2\) are mediated by EP\(_2\) and EP\(_3\) receptors. We found no evidence for the expression of EP\(_1\) or EP\(_2\) receptors in the rat afferent arteriole. In contrast, Morath et al\(^24\) recently reported that in the human kidney, the afferent arteriole stains positively for all 4 EP receptor subtypes. One obvious explanation of this difference is the possibility of species-dependent variability in EP receptor expression. Another possibility is that EP\(_2\) and EP\(_3\) mRNAs are present, but at levels that were below the detection limits in our assay. However, we found the pharmacological attributes of the responses we observed to be consistent with the results of the RT-PCR assays in that each approach suggested a primary role of EP\(_2\) and EP\(_3\) receptors.

We found message for all 3 known splice variants of the rat EP\(_1\) receptor in the rat afferent arteriole. The variable region of the EP\(_1\) receptor subtypes is confined to the intracellular carboxylic tail and determines G protein coupling.\(^24\) Because the sequence is identical from the amino-terminus through the seventh transmembrane domain, including the external portion of the receptor, the EP\(_1\) \(\alpha, \beta,\) and \(\gamma\) isoforms are thought to have similar ligand-binding characteristics.\(^3\) All 3 rat EP\(_1\) receptor isoforms can couple to G\(_{\alpha_i}\); EP\(_{\alpha_i}\) can also couple to G\(_{\alpha_q}\) and can stimulate adenyl cyclase.\(^3,16,34\) However, sulprostone had no vasodilatory effect on the afferent arteriole (Figure 6), which argues against a role of EP\(_1\) receptors in the PGE\(_2\)-induced afferent arteriolar vasodilation. A fourth vari-
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Attenuating the EP4-mediated vasodilation. Activation of EP3 duct by a similar mechanism.33 Thus, the EP3 receptor reduce cAMP-dependent water flux in the cortical collecting 

taining adequate PGC during conditions of low renal perfu-

Although the role of the vasodepressant action of PGE2 is widely appreciated, the significance of the PGE2-induced vasoinhibition is not understood. In our study, PGE2 elicited a pronounced vasoconstriction in the presence of Ang II but only a modest response when administered alone (Figure 2). Similarly, full expression of the sulprostone-induced vasoinhibition required pretreatment with both Ang II and PGE2. We interpret these observations as suggesting that EP3 receptor activation alone is not sufficient to cause vasoconstriction but rather promotes vasoconstriction by counteracting EP2 signaling and reversing the PGE2-induced vasodilation. Thus, at low concentrations, PGE2 stimulates EP2 receptors, activates Gαs and adenylyl cyclase, and elevates cAMP. At higher concentrations or under conditions in which the EP2 receptor expression is upregulated, PGE2 concurrently stimulates EP2 receptors. EP2 receptor activation inhibits adenylyl cyclase and decreases cAMP via Gαs; thereby attenuating the EP2-mediated vasodilation. Activation of EP3 receptors by PGE2 and sulprostone have been shown to reduce cAMP-dependent water flux in the cortical collecting duct by a similar mechanism.33 Thus, the EP3 receptor pathway could provide a functional antagonism of the vaso-

vasoconstrictor actions of PGE2. At the moment, one can only speculate on the potential significance of this interaction. However, it is important to note that although the preglomer-

ular vasodepressor actions of PGE2 are beneficial in main-


Acknowledgments

This work was supported by an operating grant from the Heart and Stroke Foundation of Alberta and the Northwest Territories. R. Loutzenhiser is a Senior Medical Scholar of the Alberta Heritage Foundation for Medical Research. The authors wish to thank Dr Mike Walsh, Dr Neal Davies, Bob Winkfein, and Cindy Sutherland for their help with the RT-PCR studies.

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doi: 10.1161/01.RES.86.6.663

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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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*In Vitro* Perfused Hydronephrotic Kidney Studies:

Male Sprague Dawley rats were used in all experiments. The *in vitro* perfused hydronephrotic rat kidney was used to examine the renal microvascular actions of PGE2. This model has been described in previous publications (1). In brief, unilateral hydronephrosis was induced by ligating the left ureter under halothane-induced anesthesia. Kidneys were harvested after 6 weeks, at which time the tubular atrophy has progressed to a stage that allows direct visualization of the renal microvasculature. The renal artery was cannulated and the hydronephrotic kidney was excised with continuous perfusion. The kidneys were perfused at a renal arterial pressure of 80 mmHg. The perfusate consisted of a modified Dulbecco's medium containing bicarbonate 30 mM, glucose 5 mM, and hepes 5 mM, and was equilibrated with 95% air/5% CO2 (pO2 150 mmHg). The perfusate was not recirculated. Temperature and pH were maintained at 37 °C and 7.4 respectively.

Initial studies were performed to determine the effects of basal prostaglandin production on angiotensin II-induced vasoconstriction. In all subsequent studies, kidneys were pre-treated with 10 μM ibuprofen to eliminate the influence of endogenous prostanooids. PGE2 (Calbiochem, La Jolla, CA) was prepared fresh from a 1.0 mM stock. Ro 20-1724 was obtained from Research Biochemicals International (Natick, MA) and was administered at a concentration of 0.5 μM. Sulprostone and 11-deoxy PGE1 were obtained from Cayman Chem. Co. (Ann Arbor, MI). Butaprost was generously provided by Dr. Kluender of Bayer Pharmaceutical Division (West Haven, CT). SC-51322 was
obtained from BIOMOL Research Laboratories (Plymouth Meeting, PA). Pertussis toxin was obtained from Sigma (St Louis MO). In the studies examining the effects of pertussis toxin, kidneys were first equilibrated with single-pass perfusion for at least one hour, then switched to a recirculating system and perfused with sterile medium (100 ml, 100 U penicillin-streptomycin/ml, GIBCO-BRL) containing either pertussis toxin (200 µg/ml) or vehicle for one hour. The kidneys were then returned to the single pass perfusion system, allowed to equilibrate and the responses to angiotensin II and PGE2 were assessed.

Vessel diameters were measured by on-line image processing. Video images were digitized (model IVG-128, Datacube) and diameters were measured at each pixel point and averaged over a 10-20 µm segment of the vessel. Measurements were taken at a rate of approximately 2 Hz and steady-state responses were averaged over approximately 3-5 minutes. Afferent arteriolar diameters were measured between the mid-point and origin from the interlobular artery. Efferent arteriolar diameters were measured within 50 microns of the glomerulus. In the studies comparing afferent and efferent arteriolar responses, paired arterioles from the same glomerulus were used. Only one data set (afferent arteriole, or one pair of afferent and efferent arterioles) was obtained from each kidney preparation.

*In Vitro* Perfused Normal Kidney Studies:

For the normal perfused kidney studies, male Sprague Dawley rats, weighing from 220 to 275 g were anesthetized with methoxyflurane. The left renal artery was cannulated *in situ* and the kidney was excised with continuous perfusion. Kidneys were then perfused *in vitro* as described above at a constant renal arterial pressure of 80 mm Hg,
monitored at the level of the renal artery. Perfusate flow was measured using a transonic flow meter (model T106, Transonic Systems, Inc.).

RT-PCR of EP mRNA Expression in Isolated Afferent Arterioles:

Adult male Sprague Dawley rats were anesthetized with halothane and the left kidney was flushed in vivo with warm DMEM. The kidney was then perfused with 37°C seaprep/seaplaque (2% in DMEM), excised and chilled (4°C), to solidify the agarose. Cortical slices (400 μm) were prepared and treated with collagenase IV, hyaluronidase IV, dispase II, and DNase I to dissociate microvessels from tubules. Six to eight arterioles were individually transferred to fresh buffer, and this procedure was repeated 3 times. RT-PCR was run on the final wash as a control to test for possible contamination (Figure 1 Online).

Arteriolar total RNA was extracted using a method adapted to small samples. Vessels were placed in 250 μl of solution containing 0.14 M NaCl, 10 mM Tris (pH 8.6), 1.5 mM MgCl2, and 0.5% Nonidet 40. An equal volume of 0.2 M Tris (pH 8.0), 25 mM EDTA, 0.3 M NaCl, 2% SDS and proteinase K (200 μg/ml) were added. After incubating at 37°C for 30 minutes, proteins were removed by phenol-chloroform extraction and total nucleic acids precipitated with 2.5 volumes of 100% ethanol. To remove genomic DNA, the samples were incubated with DNase I (2 μg/ml, 37°C for 1 hour), then extracted with phenol-chloroform (to remove DNase). Total RNA was precipitated by adding 2 μl seeDNA (Amersham, England) and 2.5 volumes of 100% ethanol. The pellet was dissolved in 10 μl TE (10 mM Tris HCl, pH 8.0 and 1 mM EDTA). The following were then added: 2μl 10x RT buffer (100 mM TRIS pH 8.3, 500 mM KCl, 15 mM MgCl2), 1
μl deoxynucleotide mixture (10 mM), 1μl DTT (100 mM), 1 μl random primer mixture, 1 μl RNAse inhibitor (GIBCO-BRL), 1 μl AMV reverse transcriptase (GIBCO-BRL), and 3 μl water. To rule out genomic contamination, a separate sample was treated as above, but without reverse transcriptase (Figure 1 Online). Reaction tubes were incubated at 42°C for 60 minutes and at 90°C for 3 minutes and then iced.

The primers for RT-PCR assays of the rat EP-receptor mRNAs were designed from the reported sequences (2-7) and are presented in Table 1. We assayed for a sequence common to all EP3 receptors (EP3βγ), in addition to sequences specific for each EP3 splice-variant. The denaturing, elongating and annealing PCR conditions used for the EP1 and EP2 primers were 94, 55, and 72 °C and 1.0, 1.0, and 1.5 minutes, respectively (40 cycles). For the EP3βγ primers, the conditions were 94, 55, and 72 °C for 1.0, 1.0 and 1.5 minutes (35 cycles). For EP3α, EP3β and EP3γ primers, the conditions were 94, 60, 72 °C for 1.0, 1.0, and 2.0 minutes, respectively (40 cycles). For the EP4 primers, the conditions were 94, 55, and 72 °C for 1.0, 1.0, and 1.5 minutes (35 cycles). A whole-kidney homogenate was used as a positive control for the EP receptors. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control and for the negative controls (sense and antisense primers:

5'CGGAGTCAACGGATTGG TCGTAT and 5'AGCCTTCTCCATGTTGTTGA AGAC (8)). PCR products were separated by electrophoresis using 2% agarose gel and were stained with ethidium bromide. The identity of the PCR products obtained using each set of primers was confirmed by DNA sequencing. When negative PCR results were obtained (i.e., for EP1 and EP2 primers), fresh Taq DNA polymerase was added after 35
cycles and the PCR was then continued to 70 cycles.

Analysis: Data are expressed as the mean values followed by the standard error of the mean. Differences between means were evaluated by ANOVA followed by Student's t test (paired or unpaired). Values of P<0.05 were considered statistically significant. For multiple comparisons, the Bonferroni correction was applied and P values <0.05/n (where n= number of comparisons) were considered significant.

REFERENCES:


Figure 1 Online:

The EP1 receptor antagonist SC-51322 did not alter the vasoconstrictor response of the afferent arteriole to sulprostone (n=5, p>0.10 for sulprostone alone versus sulprostone + 10 μM SC51322).
Table 1  EP Receptor Primers

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Primer Sequence</th>
<th>Position</th>
<th>Reference</th>
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<tbody>
<tr>
<td>EP1 sense</td>
<td>5'TTGGTGTGCTTCGCTGTATACTG</td>
<td>285-306</td>
<td>(2)</td>
</tr>
<tr>
<td>antisense</td>
<td>5'CCAGTGATGCTCGATGTCATGG</td>
<td>534-555</td>
<td></td>
</tr>
<tr>
<td>EP2 sense</td>
<td>5'GACTACACGCACCTTCATCCTAC</td>
<td>277-298</td>
<td>(3)</td>
</tr>
<tr>
<td>antisense</td>
<td>5'CTTGTCACGTCCTGGCTCTTAG</td>
<td>378-399</td>
<td></td>
</tr>
<tr>
<td>EP3αβγ sense</td>
<td>5'TGCCAGCCACATGAAGACTCGC</td>
<td>426-447</td>
<td>(4)</td>
</tr>
<tr>
<td>antisense</td>
<td>5'ATAGCGGCCTCCTCGGTGGATCC</td>
<td>754-775</td>
<td></td>
</tr>
<tr>
<td>EP3-sense</td>
<td>5'ATGGGGGATCATGTGTGTACTG</td>
<td>911-929</td>
<td>(3, 5,6)</td>
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<tr>
<td>α antisense</td>
<td>5'TGGAAGCATAGTTGGTGATGG</td>
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</tr>
<tr>
<td>β antisense</td>
<td>5'AAATCCGCTTTACAGTTGTTC</td>
<td>1011-1031</td>
<td></td>
</tr>
<tr>
<td>γ antisense</td>
<td>5'TAGACAATGAGATGGCCCTGC</td>
<td>1050-1069</td>
<td></td>
</tr>
<tr>
<td>EP4 sense</td>
<td>5'CCTTCTTCGGAAGACTGTGCTC</td>
<td>999-1020</td>
<td>(7)</td>
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<tr>
<td>antisense</td>
<td>5'CAGAAGATGTCCTCCGACTCTC</td>
<td>1112-1133</td>
<td></td>
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