Glomerular Cytochrome P-450 and Cyclooxygenase Metabolites Regulate Efferent Arteriole Resistance

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Abstract—Bradykinin dilates efferent arterioles via release of efferent arteriole epoxyeicosatrienoic acids when perfused retrograde (no glomerular autacoids). However, when efferent arterioles are perfused orthograde through the glomerulus, bradykinin-induced dilatation is caused by a balance between: (1) the glomerular vasoconstrictor 20-hydroxyeicosatetraenoic acid and vasodilator prostaglandins, and (2) epoxyeicosatrienoic acids from the efferent arteriole and possibly the glomerulus. However, the role of 20-hydroxyeicosatetraenoic acid has only been studied with a cyclooxygenase inhibitor, which may artificially enhance its production by shunting arachidonic acid into the cytochrome P450 pathway. We hypothesized that in the absence of cyclooxygenase inhibition, bradykinin induces release of 20-hydroxyeicosatetraenoic acid from the glomerulus, which blunts the vasodilator effect of bradykinin; and that prostaglandins released from glomeruli in response to bradykinin are generated by cyclooxygenase-1. Rabbit efferent arterioles preconstricted with norepinephrine were perfused orthograde from the end of the afferent arteriole. Bradykinin was added to the perfusate with or without a 20-hydroxyeicosatetraenoic acid antagonist (20-HEDE), epoxyeicosatrienoic acid synthesis inhibitor (MS-PPOH), and/or cyclooxygenase-1 (SC-58560) or cyclooxygenase-2 inhibitor (NS-398). Bradykinin-dependent dilatation was enhanced by 20-HEDE but blunted by MS-PPOH. When the inhibitors were present, bradykinin-induced dilatation was abolished by blockade of cyclooxygenase-1 but not cyclooxygenase-2. We concluded that: (1) in the absence of cyclooxygenase inhibitors, bradykinin causes the release of a glomerular vasoconstrictor (20-hydroxyeicosatetraenoic acid) that antagonizes the vasodilator effect of epoxyeicosatrienoic acids released from the efferent arteriole and perhaps from the glomerulus, and (2) bradykinin-induced vasodilatation is caused by the release of epoxyeicosatrienoic acids from the efferent arteriole and glomerular metabolites of cyclooxygenase-1. (Hypertension. 2005;46:1175-1179.)

Key Words: glomerulus ■ cyclooxygenase-1 ■ 20-hydroxyeicosatetraenoic acid ■ epoxyeicosatrienoic acid ■ renal vascular resistance

Renal kinins are released at both the luminal and basolateral sides of the nephron by kallikrein, which is located mainly in the connecting tubule. Kinins released from this segment could stimulate the release of vasoactive autacoids from the glomerulus, which in turn could regulate the resistance of the downstream efferent arteriole (Ef-Art).1 Intrarenal bradykinin (BK) infusion decreases renal vascular resistance, thereby increasing renal blood flow.2 BK B₂ receptors are located in both the glomerulus and the Ef-Art and are involved in the vasodilator effect of BK on the Ef-Art via epoxyeicosatrienoic acids (EETs).1,3,4 In the glomerulus, activation of the B₂ receptor by BK is known to stimulate the phospholipase A₂ pathway, generating arachidonic acid. Glomerular cyclooxygenase (COX) metabolizes arachidonic acid to prostaglandin (PG) H₂. This is followed by further metabolism into bioactive PG E₂ and PG I₂ via PGE synthases and prostacyclin synthase, respectively. Both PGE₂ and PGI₂ have been shown to have a vasodilator effect on renal microvessels.5–9

Previously, we determined that when Ef-Arts are perfused retrograde, bypassing the glomerulus, neither nitric oxide synthase inhibitors nor COX inhibitors alter BK-induced Ef-Art dilatation.10 However, the vasodilator effect of BK was completely blocked by inhibiting the synthesis of EETs, demonstrating that these arachidonic acid metabolites of the cytochrome P450 pathway totally account for BK-induced dilatation. In contrast, when Ef-Arts were perfused orthograde via the glomerulus, inhibition of COX with indomethacin blocked the Ef-Art dilatation caused by BK. In the presence of indomethacin, 20-HEDE, an antagonist of hydroxyeicosatetraenoic acid (20-HETE), completely restored the vasodilator effect of BK when Ef-Arts were perfused orthograde. Thus we concluded that the glomerulus releases both vasodilator prostaglandins and the vasoconstrictor 20-
HETE. However, inhibiting COX may shunt arachidonic acid normally metabolized by COX into the cytochrome P450 pathway, thereby enhancing production of 20-HETE and EETs.\textsuperscript{16} Furthermore, it was not clear from our previous reports whether glomerular prostaglandins are released via the COX-1 or COX-2 pathway. Here we tested whether in the absence of COX inhibition the vasconstrictor 20-HETE released by the glomerulus plays a role in regulating Ef-Art tone. In addition, we examined whether the vasodilator prostaglandins released by the glomeruli in response to BK are generated by COX-1 or COX-2.

**Methods**

We used methods similar to those described previously to isolate the Ef-Art with glomerulus and afferent arteriole (Af-Art) intact.\textsuperscript{10} Male New Zealand White rabbits (Covance Research Products; Kalamazoo, Mich; 1.5 to 2.1 kg) were fed standard rabbit chow (Ralston Purina) and given tap water ad libitum. All experiments were approved by the Henry Ford Health System Institutional Animal Care and Use Committee (IAUC) and were conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. Rabbits were anesthetized with ketamine plus xylazine (50 and 10 mg/kg IM) and sodium pentobarbital (30 mg/kg IV), and heparin (500 U IV) was injected to block coagulation. The kidneys were sliced longitudinally along the cortex-medullary axis. Slices were placed in ice-cold minimum essential medium (MEM) (Gibco) containing 5% BSA (Intergen), and a single superficial Ef-Art with glomerulus and Af-Art intact was microdissected under a stereomicroscope. Using a micropipette, the preparation was transferred to a temperature-regulated chamber mounted on the stage of an inverted microscope with Hoffman modulation, and the Ef-Art was perfused with oxygenated MEM containing 5% BSA via the glomerulus from the end of the Af-Art (orthograde perfusion). The perfusion rate was adjusted to maintain an average intraluminal pressure of 50 mm Hg as measured by Landis’ technique. Thus, because the perfusate traveled through the glomerulus, hormonal factors released by the glomerulus were able to reach the downstream Ef-Art. The bath, which was exchanged continuously, was similar to the perfusate except that it contained 0.15% BSA.

Microdissection and cannulation were completed within 90 minutes at 3°C, after which the bath was gradually warmed to 37°C for the rest of the experiment. Once temperature was stable, a 30-minute equilibration period was allowed before any measurements were taken. Images of the Ef-Art were displayed and recorded at magnifications up to 1980×. Diameter was measured with an image analysis system.

BK and norepinephrine were purchased from Sigma. Based on our previous study, concentrations of 20-HEDE and MS-PPOH were 1 μmol/L.\textsuperscript{10} A stock solution of N-[2-(cyclohexyloxy)-4-nitrophenyl]-methanesulfonamide (NS-398; Cayman) was prepared in dimethyl sulfoxide (DMSO). The final DMSO concentration was 0.01%, which had no effect by itself. NS-398 reportedly has a half-maximal inhibitory concentration \( IC_{50} = 0.05 \mu \text{mol/L} \) for human COX-2 and 0.15 μmol/L for ovine COX-2.\textsuperscript{11,12} SC-58560 was a generous gift from Pfizer and reportedly has an \( IC_{50} = 0.0048 \) for recombinant human COX-1.\textsuperscript{13}

**Protocols**

Perfused preparations were first pretreated with norepinephrine (3 μmol/L) to reduce basal Ef-Art diameter by \( \approx 40\% \), and then BK (0.01 to 10 nmol/L) was added to the lumen to generate a dose-response curve. Luminal diameter at the site of maximum response was measured immediately before adding BK and 10 minutes after adding each dose of BK. After a 20-minute washout period, a second BK dose-response curve was generated in the presence of a 20-HETE antagonist (20-HEDE), an EET synthesis inhibitor (MS-PPOH), and/or COX inhibitors (SC-58560 for COX-1 and NS-398 for COX-2).

**Statistics**

Values are expressed as mean±SE. ANOVA was used to assess overall change, with dose as the repeated measure. Each dose was compared with norepinephrine baseline using a paired \( t \) test. When significant interactions were found between dose and treatment group, paired \( t \) tests were performed to determine which dose(s) altered luminal diameter. Hochberg’s method was used to adjust the \( \alpha \) level of significance for multiple comparisons. \( P<0.05 \) was considered significant.

**Results**

First, we studied whether the response to BK was stable over time. In norepinephrine-preconstricted Ef-Arts, basal diameter decreased by 41.3% (from 12.6±0.5 to 7.4±0.4 μm; \( n=5 \)); 0.01 nmol/L BK had no effect; however, 0.1, 1, and 10 nmol/L BK increased diameter to 9.6±0.4, 10.9±0.3, and 12.1±0.5 μm, respectively. The second dose-response curve was similar; Ef-Art diameter increased from 7.1±0.5 to 9.4±0.3, 10.6±0.3, and 12.0±0.4 μm at 0.1, 1 and 10 nmol/L, respectively. Thus no tachyphylaxis was observed.

To test whether BK stimulates the release of glomerular 20-HETE in the absence of COX inhibition, we studied the effect of a selective 20-HETE antagonist, 20-HEDE, on BK-induced dilatation of the Ef-Art perfused orthograde through the glomerulus. 20-HEDE (1 μmol/L) added to the lumen and bath did not alter basal diameter; however, it significantly enhanced BK-induced dilatation (\( n=9 \); \( P<0.02 \), untreated versus 20-HEDE) (Figure 1). These data suggest that 20-HETE released from the glomerulus in response to BK plays a role in constricting the Ef-Art in the absence of COX inhibition.

To study the role of EETs in BK-induced dilatation in the absence of COX inhibition, we examined the effect of adding the specific EET synthesis inhibitor MS-PPOH (1 μmol/L) to the lumen and bath. In the presence of MS-PPOH, BK-induced dilatation of the Ef-Art was significantly attenuated (\( n=6 \); \( P<0.02 \), untreated versus MS-PPOH) (Figure 2). We also examined the effect of MS-PPOH in the presence of 20-HEDE. Adding 1 μmol/L MS-PPOH to the lumen and bath together with 20-HEDE significantly attenuated the vasodilator effect of BK (\( n=6 \); \( P<0.01 \), with versus without MS-PPOH) (Figure 3). Taken together, these data indicate that EETs mediate at least part of the vasodilator effect of BK in the absence of COX inhibition.

Previously, we have shown that prostaglandins released from the glomerulus also partially mediate the effect of BK on the Ef-Art. However, we did not study whether COX-1 or COX-2 was responsible. Consequently, we next tested the effect of a selective COX-1 inhibitor, SC-58560, on BK-induced dilatation. Ef-Arts were perfused orthograde in the presence of 20-HEDE and MS-PPOH. When 1 μmol/L SC-58560 was added in the presence of 20-HEDE and MS-PPOH, BK did not increase luminal diameter (\( n=6 \); \( P<0.001 \), with versus without SC-58560) (Figure 4). To test whether glomerular prostanoids released by COX-2 participate in BK-induced dilatation, we added 1 μmol/L NS-398 (a selective COX-2 inhibitor) to the lumen and bath in the presence of 20-HEDE and MS-PPOH. BK-induced dilatation...
of the Ef-Arts showed no difference with or without NS-398 (n=6). These data suggest that when the glomerulus is stimulated with BK, it releases vasodilator prostanoids via the COX-1 but not the COX-2 pathway.

Discussion

Previously, we found that when Ef-Arts were perfused retrograde, EETs produced by cytochrome P450 enzymes totally accounted for BK-induced dilatation. We also found that when the Ef-Art was perfused orthograde BK also induced dilatation, but this effect was completely blocked by a COX inhibitor and restored by a 20-HETE antagonist. Thus, we concluded that the glomerulus releases both a vasoconstrictor (20-HETE) and vasodilators (prostaglandins) that have an antagonistic and a synergistic effect, respectively, on BK-induced dilatation of the Ef-Art. However, inhibiting the COX pathway may shunt arachidonic acid normally metabolized by COX into the cytochrome P450 pathway, thereby increasing the contribution of cytochrome P450 metabolites to Ef-Art regulation. Thus we investigated whether, in the absence of COX inhibition, cytochrome P450 metabolites of arachidonic acid play a significant role in regulating Ef-Art tone. For this we used a selective 20-HETE antagonist, 20-HEDE, which blocks the vasoconstrictor response to 20-HETE in renal arterioles. We also used a selective inhibitor of EET production, MS-PPOH, that has been shown to abolish BK dilatation of juxtamedullary Af-Arts. We found that 20-HEDE did not alter basal diameter, but significantly enhanced BK-induced dilatation of the Ef-Arts. We also found that both with and without 20-HEDE, MS-PPOH reduced but did not abolish BK-induced dilatation of the Ef-Art. Taken together, these data indicate that cytochrome P450 arachidonic acid metabolites released from the glomerulus (20-HETE, constrictor) and Ef-Art (EETs, dilators) play opposing roles in the vasodilator effect of BK on the Ef-Art.

Previously, we found that when Ef-Arts are perfused retrograde, inhibition of either nitric oxide synthase or COX does not alter the vasodilator effect of BK, indicating that Ef-Arts do not release nitric oxide or prostaglandins in response to BK. In the present study, we found that when Ef-Arts were perfused orthograde in the presence of 20-HEDE and MS-PPOH, the vasodilator effect of BK was completely blocked by a selective COX-1 inhibitor (SC-58560), whereas COX-2 inhibition had no such effect. We conclude that glomerular COX-1 releases vasodilator prostaglandins in response to BK that partially mediate dilatation of the Ef-Art when it is perfused in a physiological manner (orthograde). The selectivity of the COX-1 inhibitor we used (SC-58560) has already been demonstrated. Using an in vitro perfused rat juxtamedullary nephron preparation, Imig and Deichmann reported that a selective COX-2 inhibitor, NS-398, did not affect the Af-Art response to angiotensin II, but enhanced the Af-Art vasoconstrictor response to norepinephrine. Based on our previous and present work, it
appears that eicosanoids both mediate and antagonize the effects of BK in the Ef-Art. The EETs and prostaglandins that mediate the vasodilator effect of BK originate in the Ef-Art and the glomeruli, respectively, whereas the vasoconstrictor 20-HETE that antagonizes the vasodilator effect of BK originates mainly in the glomerulus. Whether EETs are also produced by the glomerulus cannot be established by our studies, because during retrograde perfusion the Ef-Art produces EETs that mediate BK-induced dilatation. In the kidney, arachidonic acid metabolites produced by cytochrome P450 enzymes play a major role in the regulation of not only renal vascular tone but also sodium transport, inflammation, and tubuloglomerular feedback.21–23 Cytochrome P450 enzymes metabolize arachidonic acid to a series of EETs and HETEs.24 20-HETE is a potent constrictor of renal arterioles.25,26 The glomerulus produces 20-HETE, dihydroxyeicosatetraenoic acids (diHETEs), and 12-HETE when incubated with arachidonic acid.24 On the other hand, EETs are vasodilators,25,26 which may be important in the control of glomerular hemodynamics. Imig et al27 demonstrated that when renal microvessels are stimulated by bradykinin, they produce EETs that in turn dilate the Af-Art. We have shown that when the Ef-Art is perfused orthograde via the glomerulus, BK induces release of 20-HETE and EETs, constrictor and dilator cytochrome P450 metabolites of arachidonic acid that mediate its effects both in the presence10 and absence of COX inhibition (this study). Thus, our data support the contention that these compounds are important regulators of the renal microcirculation.

COX enzymes are necessary for synthesis of prostaglandins from arachidonic acid. There are 2 different isoforms of COX, referred to as COX-1 and COX-2, which are encoded by different genes.28 Recent studies have demonstrated that both isoforms are present in the kidney.29 COX-1 is constitutively expressed in the glomerulus, the thick ascending limb of Henle’s loop, and the collecting duct,30 and is thought to be responsible for continuous generation of prostaglandins that regulate renal hemodynamics and tubular transport.31 Recent studies showed that PGE2 EP2 and EP4 receptors are located in both cortical preglomerular resistance vessels and outer medullary vasa recta bundles,32,33 and PGI2 IP receptor mRNA has been detected in both Af-Arts and vasa recta.34,35 These receptors might participate in the vasodilator effect of COX-1–derived prostaglandins on the Ef-Art. Consistent with previous reports, we conclude from our present data that the vasodilator prostaglandins that mediate BK-induced dilatation of the Ef-Art are produced by COX-1 rather than COX-2.

Perspectives

BK is considered to be an endothelial nitric oxide-dependent vasodilator; however, clearly in the Ef-Art its vasodilator effect is mediated not by nitric oxide but rather by a balance between arachidonic acid metabolites of cytochrome P450 and COX-1 that are vasoconstrictors and vasodilators. The eicosanoids released by the glomerulus and Ef-Art may be
important in regulating not only Ef-Art tone but also glomerular filtration rate by altering resistance in the Ef-Art and consequently glomerular capillary pressure. One could speculate that eicosanoids released by the glomerulus and Ef-Art may regulate not only Ef-Art resistance but also the downstream renal circulation, and thereby participate in the regulation of water and sodium excretion. The present studies illustrate the complexity of the pathways that regulate the renal microcirculation and suggest that inhibition of eicosanoid synthesis may have unexpected effects on renal function.

Acknowledgments

This work was supported by grants HL28982 and GM-31278 from the National Institutes of Health.

References

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Hypertension. 2005;46:1175-1179; originally published online October 17, 2005; doi: 10.1161/01.HYP.0000187531.93389.63

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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