Afferent Arteriolar Vasodilation to the Sulfonimide Analog of 11,12-Epoxyeicosatrienoic Acid Involves Protein Kinase A


Abstract—The current study determined the contribution of protein kinase-A (PKA) and protein kinase-G (PKG) to the vasodilation elicited by the N-methylsulfonimide analog of 11,12-epoxyeicosatrienoic acid (11,12-EET). Experiments were performed, in vitro, using the juxtamedullary nephron preparation combined with videomicroscopy. The response of afferent arterioles to the sulfonimide analog of 11,12-EET, was determined before and after inhibition of PKA, PKG, or guanylyl cyclase. Afferent arterioles, preconstricted with 0.5 μmol/L norepinephrine, averaged 18 ± 1 μm (n = 25) at a renal perfusion pressure of 100 mm Hg. Superfusion with 0.01 to 100 nmol/L of the 11,12-EET analog caused a graded increase in diameter of the afferent arteriole. Vessel diameter increased by 11 ± 1% and 15 ± 1%, respectively, in response to 10 and 100 nmol/L of the 11,12-EET analog. The afferent arteriolar response to 10 and 100 nmol/L of the 11,12-EET analog was significantly attenuated during inhibition of PKA with 10 μmol/L H-89 (n = 7) or 5 μmol/L myristilated PKI (n = 6), such that afferent arteriolar diameter increased by only 5 ± 2% and 2 ± 1%, respectively, in response to 100 nmol/L of the 11,12-EET analog. In contrast, the afferent arteriolar vasodilatory response to the 11,12-EET analog was unaffected by PKG or guanylyl cyclase inhibition. In the presence of 200 μmol/L histone H2B (n = 5) or 10 μmol/L ODQ (n = 7), the afferent arteriolar diameter increased by 16 ± 3% and 12 ± 2%, respectively, in response to 100 nmol/L of the 11,12-EET analog. These results demonstrate that activation of PKA is an important mechanism responsible for the afferent arteriolar vasodilation elicited by the sulfonimide analog of 11,12-EET.

(Hypertension. 1999;33[part II]:408-413.)

Key Words: metabolites, cytochrome P450 ■ kidney ■ endothelium-derived hyperpolarizing factor ■ arterioles ■ cyclic adenosine monophosphate ■ cyclic guanosine monophosphate

Epoxyeicosatrienoic acids (EET) produced by the kidney affect both renal blood flow and tubular transport function. In this regard, renal enzymatic epoxygenase activity in some strains of rats on a high-salt diet and has been shown to be altered during the development of hypertension. Thus, renal epoxygenase metabolites have been implicated in the maintenance of water and electrolyte balance.

More recently, EETs have been demonstrated to mediate agonist-induced endothelium-dependent vasodilation and have been considered an endothelium-derived hyperpolarizing factor (EDHF). In the renal circulation, EDHF appears to mediate a large portion of the vasodilatory response to bradykinin and an endothelial-derived cytochrome P450 metabolite of arachidonic acid has been implicated as an EDHF. We previously demonstrated that 11,12-EET which is designed to resist esterification and tubular oxidation while retaining full biological activity, is an EDHF. Vasodilation and activation of vascular smooth muscle channels can occur via stimulation of cAMP-dependent and cGMP-dependent protein kinases. Therefore, the present study determined the contribution of cAMP- and cGMP-dependent protein kinases in mediating the 11,12-EET effect on afferent arteriolar diameter. Experiments were performed with the newly synthesized N-methylsulfonimide analog of 11,12-EET which is designed to resist esterification and β-oxidation while retaining full biological activity. Preglomerular vascular responses to the 11,12-EET analog and the involvement of protein kinases were determined utilizing the in vitro perfused juxtamedullary nephron preparation.

Methods

Vascular Preparation

Experiments were performed on male Sprague-Dawley rats (Charles River Laboratories, Wilmington, Mass) weighing an average of...
Afferent Arteriolar Vasodilation to the 11,12-EET Analog of 11,12-EET

Synthesis of the N-Methylsulfonimide Analog of 11,12-EET

(±)-11,12-EET was prepared as previously described.19 (±)-11,12-EET (18 mg, 0.05 mmol) and N-hydroxysuccinimide (7.1 mg, 0.06 mmol) were azeotropically dried with benzene (3×20 mL), then dissolved in dry tetrahydrofuran (1 mL) and cooled to 0°C. To this was added dicyclohexylcarbodiimide (12.5 mg, 0.06 mmol) and the solution vigorously stirred. After 12 hours at room temperature, the solvent was removed in vacuo and the crude product purified by SiO2 column chromatography using EtOAc/hexane (30:70), to give the corresponding N-hydroxysuccinimide ester as a gum (21 mg, 92%). Thin-layer chromatography (TLC, SiO2): 30% EtOAc in hexane, RF ≈ 0.3; 1H nuclear magnetic resonance (NMR, 250 MHz, CDCl3); δ 0.90 (t, J = 6.9 Hz, 3H), 1.21 to 1.42 (m, 6H), 1.85 to 1.90 (m, 2H), 2.00 to 2.15 (m, 2H), 2.17 to 2.30 (m, 4H), 2.37 to 2.45 (m, 2H), 2.60 (t, J = 7.4 Hz, 2H), 2.88 to 2.92 (m, 6H), 2.92 to 2.99 (m, 2H), 5.25 to 5.50 (m, 6H).

The above active ester (21 mg, 0.05 mmol), methanesulfonimide (47.6 mg, 0.5 mmol), and 4-dimethylaminopyridine (6.1 mg, 0.05 mmol) were dried in vacuo (0.1 mm Hg) for 2 hours, mixed with anhydrous hexamethyldisilazane (0.05 mL), and heated at 90°C under an argon atmosphere. After 1.5 hours, the reaction mixture was cooled and partitioned between water and EtOAc. The aqueous layer was extracted twice with more EtOAc. The combined organic extracts were dried over MgSO4, evaporated in vacuo, and the residue purified via preparative TLC using EtOAc/hexane (1:1) to afford the N-methylsulfonimide analog of (±)-11,12-EET (11.6 mg, 58%) as a colorless oil. TLC (SiO2): EtOAc/hexane, (1:1) RF ≈ 0.4; 1H NMR (250 MHz, CDCl3); δ 0.9 (t, J = 6.8 Hz, 3H), 1.22 to 1.40 (m, 6H), 1.72 to 1.80 (m, 2H), 1.95 to 2.90 (m, 4H), 2.20 to 2.35 (m, 4H), 2.40 to 2.52 (m, 2H), 2.62 to 2.70 (m, 1H), 2.89 to 3.10 (m, 3H), 3.32 (t, 3H), 5.30 to 5.62 (m, 6H).

Afferent Arteriolar Vasodilation to the 11,12-EET Analog and the Involvement of Protein Kinases

Stock solutions of the N-methylsulfonimide analog of 11,12-EET in ethanol were kept in sealed vials and stored under nitrogen at −80°C until the experiment. Immediately before use, the stock solution of the 11,12-EET analog was added to the superfusion solution. The final concentration of the ethanol vehicle was <0.05% (vol/vol). The diameter response to the 11,12-EET analog was maximal by 1 to 2 minutes and sustained over a 15-minute period. All subsequent experiments monitored the vascular diameter over a 5-minute period. An afferent arteriole was chosen for experiments designed to determine the involvement of protein kinases in the vasodilation to the sulfonimide analog of 11,12-EET. Measurements of afferent arteriolar diameters were made at least 50 μm from any branch points. Vessels were preconstricted with a submaximal concentration of norepinephrine (0.5 μmol/L; Sanofi/Winthrop Pharm). Administration of norepinephrine to the blood perfusate decreased the diameter of afferent arterioles from 22±1 to 18±1 μm (n = 41). After preconstriction with norepinephrine, the afferent arteriolar response to increasing concentrations of the N-methylsulfonimide analog of 11,12-EET (0.01 to 100 nmol/L) was determined. After the control dose response to the 11,12-EET analog was obtained, the protein kinase inhibitors were added to the perfusate and superfusate for 20 minutes to ensure complete blockade. The afferent arteriolar dose response to the 11,12-EET analog was repeated in the presence of protein kinase A (PKA) inhibitor H-89 (10 μmol/L; Biomol) or myristolated PKI (14–22) amide (5 μmol/L; Biomol). The response of the afferent arterioles to the 11,12-EET analog was also determined before and after inhibition of protein kinase G (PKG) with histone H2B (29–35) (200 μmol/L; Biomol) or guanyl cyclase with ODQ (10 μmol/L; Biomol). No differences in repeat afferent arteriolar responses to the N-methylsulfonimide analog of 11,12-EET (n = 4) were observed in time control experiments. Additional experiments were performed to determine the effectiveness of the protein kinase inhibitors for blocking protein kinase activity. The ability of H-89 or myristolated PKI to inhibit the afferent arteriolar vasodilatory response to the cell permeable cAMP analog, 8-brorno-cAMP was determined. After the afferent arteriolar response to 8-brorno-cAMP (1 nmol/L to 100 μmol/L; Biomol) was determined, the PKA inhibitor H-89 or myristolated PKI was administered to the perfusate and superfusate and the response to 8-brorno-cAMP redetermined. Likewise, the ability of the PKG inhibitor histone H2B to inhibit the afferent arteriolar vasodilatory response to the cell permeable cGMP analog, 8-brorno-cGMP (1 nmol/L to 100 μmol/L; Biomol) was determined.

Statistics

Data are presented as mean±SEM. Significance of differences in mean values for the dose-response effect was evaluated by analysis of variance for repeated measures followed by Duncan’s multiple range test. A value of P < 0.05 was considered statistically significant.

Results

Effect of PKA Inhibitors on the Afferent Arteriolar Response to the 11,12-EET Analog

Initial studies were performed comparing the vascular activity of 11,12-EET and the N-methylsulfonimide analog of 11,12-EET on afferent arteriolar diameter. Both 11,12-EET and the sulfonimide analog of 11,12-EET elicited similar vasodilatory responses. Afferent arteriolar diameter averaged 18±2 μm (n = 3) and increased by 11±2% and 15±3% in response to 10 and 100 nmol/L of the 11,12-EET analog. Likewise, afferent arteriolar diameter increased by 12±3% and 17±4% in response to 10 and 100 nmol/L 11,12-EET.

Figure 1 depicts the effect of the PKA inhibitor, H-89, on the afferent arteriolar vasodilatory response to the 11,12-EET analog. Afferent arteriolar diameter increased after superfusion with the 11,12-EET analog and reached a steady state within 2 minutes. In the presence of the PKA inhibitor H-89,
the afferent arteriolar vasodilation in response to the sulfonimide analog of 11,12-EET was significantly attenuated. The effect of PKA inhibition on the steady state responses to the 11,12-EET analog is presented in Figure 2. The vasodilatory response to 100 nmol/L of the 11,12-EET analog averaged 13±2% and was significantly attenuated in the presence of H-89 and averaged 5±2%. Likewise, the increase in the afferent arteriolar diameter to 100 nmol/L of the 11,12-EET analog averaged 15±4% and was significantly attenuated by the PKA inhibitor, myristolated PKI. In the presence of myristilated PKI, 100 nmol/L of the 11,12-EET analog increased the afferent arteriolar diameter by only 2±1%.

To control for the possibility that PKA inhibition might nonspecifically interfere with microvascular vasodilatory responses, we confirmed the ability of these arterioles to vasodilate in response to acetylcholine. Acetylcholine (1 μmol/L), administered at the end of the experiment, increased afferent arteriolar diameter by 17±2% (n=2) and 21±5% (n=2) in the presence of H-89 and myristolated PKI, respectively.

Effect of PKG and Guanylyl Cyclase Inhibition on the Afferent Arteriolar Response to the 11,12-EET Analog

Figure 3 presents the effect of the PKG inhibitor (left panel) and the guanylyl cyclase inhibitor, ODQ (right panel), on the afferent arteriolar response to N-methylsulfonimide analog of 11,12-EET. Afferent arteriolar diameter averaged 19±1 μm (n=12) and increased by 15±1% in response to superfusion of 100 nmol/L of the 11,12-EET analog. The PKG inhibitor, histone H2B, did not significantly alter the vasodilation and afferent arteriolar diameter increased by 15±3% in response to 100 nmol/L of the 11,12-EET analog during PKG inhibition. The afferent arteriolar response to the sulfonimide analog of 11,12-EET was not altered by the guanylyl cyclase inhibitor ODQ. In the presence of ODQ, afferent arteriolar diameter increased by 12±2% in response to 100 nmol/L of the 11,12-EET analog.

Effect of Protein Kinase Inhibitors on the Afferent Arteriolar Vasodilation Response to 8-Bromo Analogs

The effect of the PKA inhibitors, H-89 and myristolated PKI, on the afferent arteriolar vasodilation to 8-bromo-cAMP is depicted in the top panel of Figure 4. 8-Bromo-cAMP dose-dependently increased afferent arteriolar diameter. The afferent arteriolar vasodilatory response to 8-bromo-cAMP was significantly attenuated in the presence of PKA inhibition. 8-bromo-cAMP (100 μmol/L) increased afferent arteriolar diameter by 11±3% before the addition of PKA inhibitors and decreased diameter by 2±2% and 1±1% in the presence of H-89 and myristolated PKI, respectively.

Experiments were also performed to determine the ability of histone H2B to inhibit the afferent arteriolar vasodilation to 8-bromo-cGMP. Afferent arteriolar diameter increased dose dependently in response to 8-bromo-cGMP (Figure 4, bottom panel). The afferent arteriolar diameter response to 10 and 100 μmol/L, 8-bromo-cGMP was 9±2% and 10±3%, respectively, under control conditions. In contrast, in the presence of histone H2B, these responses were attenuated to 2±2% and 1±1%, respectively.
EETs are synthesized by endothelial cells, activate vascular smooth muscle K⁺ channels, hyperpolarize vascular smooth cells, and vasodilate blood vessels. Thus, EETs have been considered an EDHF. The major epoxygenase metabolites of arachidonic acid produced in the kidney are 11,12-EET and 14,15-EET. Renal microvessels and microsomes prepared from these vessels metabolize arachidonic acid to 11,12-EET and 14,15-EET and their corresponding diols. Recently, it has been suggested that 11,12-EET is the EDHF that mediates a large proportion of the bradykinin-induced vasodilation in coronary and renal arteries. 11,12-EET elicits endothelium- and cyclooxygenase-independent vasodilation of the preglomerular vasculature, whereas the epoxide hydrolase metabolite 11,12-dihydroxyeicosatetraenoic acid did not significantly affect afferent arteriolar tone. The vasodilation of larger caliber renal arteries by 11,12-EET was significantly attenuated by K⁺ channel inhibitors. Likewise, 11,12-EET increased the outward K⁺ current of the large conductance KCas channel active in renal arterial vascular smooth muscle cells. In these experiments, the large conductance KCas channel activity was unaltered in renal arterial vascular smooth muscle cells exposed to 11,12-EET on the cytoplasmic or external face of excised membrane patches. This suggests that cytoplasmic signal transduction factors are required for activation of K⁺ channels by 11,12-EET. The results of the present study demonstrate that vasodilation of afferent arterioles by the sulfonimide analog of 11,12-EET requires activation of the cAMP-dependent protein kinase pathway and suggest that PKA participates as a cellular signaling factor for 11,12-EET to activate K⁺ channels.

The ability of 11,12-EET to relax vascular smooth muscle and control organ blood flow is not limited to the renal vasculature. However, the ability of EETs to vasodilate does appear to depend on the vasculature from which the vessels are isolated. EETs do not affect cell membrane potential or cause relaxation of internal carotid arteries isolated from guinea pig, whereas coronary and cerebral arteries generate epoxygenase metabolites and, like the renal vasculature, 11,12-EET causes vasodilation. The vasodilatory action of 11,12-EET on the cat cerebral vasculature is associated with activation of vascular smooth muscle cell KCas channels. In contrast, the 11,12-EET-induced vasodilation of the newborn pig cerebral circulation requires an intact prostanoid system and prostacyclin receptor activation. In freshly isolated coronary artery smooth muscle cells, 11,12-EET causes membrane hyperpolarization and appears to be selective for the KCas channel since the delayed rectifier K⁺ (Kdrf) channels were unaffected by 11,12-EET. Interestingly, the prostaglandin I₂ analog iloprost increases the activity of the Kdrf channels without affecting the KCas channels in the coronary vasculature. Activation of the coronary artery KCas channels by 11,12-EET appears to be due to activation of a GTP binding protein, G, and depends on cytoplasmic signaling factors.

Although G, can activate adenyl cyclase and cause PKA-dependent phosphorylation of the KCas channel, bovine coronary artery cAMP levels were not significantly affected by incubation with 11,12-EET. This finding is not consistent with the observation of the present study that the...
11,12-EET analog-mediated vasodilation of the afferent arteriole is greatly attenuated by 2 chemically distinct inhibitors of PKA. One possible explanation for this discrepancy could be that the sensitivity of the cAMP assay was not sufficient to allow for detection of physiologically significant increases in activity. 11,12-EET activation of the cAMP-dependent pathway has been observed in heart cells. Ventricular myocytes incubated with 11,12-EET did significantly elevate intracellular cAMP levels and in this cell type 11,12-EET may act to modulate L-type Ca\(^{2+}\) channel current. Another possibility is that the vascular tissue cAMP measurements have been done on larger caliber vessels that appear to be less sensitive to the vasorelaxant effects of EETs\(^1\) and therefore may not utilize the cAMP pathway to the same extent as smaller caliber vessels. Further studies are required to clearly delineate the cellular-signaling steps responsible for the afferent arteriolar vasodilation in response to 11,12-EET.

Recent studies have shown that PKG can be the mediator of not only cGMP-dependent but also cAMP-dependent vasodilators.\(^8\) The present results demonstrate that inhibition of PKG or guanyyl cyclase inhibition.\(^39\) Likewise, epoxygenase metabolites, when incubated with bovine coronary arteries, significantly decreased tissue cGMP content.\(^13\) In addition, brefeldin A inhibited the EDHF-mediated vasorelation and production of EDHF elicited by bradykinin in porcine coronary arteries but did not affect the accumulation of cGMP.\(^40\) Thus, the results of the current study and those of previous studies do not support a role for the cGMP-dependent protein kinase pathway in the vasodilatory response to 11,12-EET.

In summary, the N-methylsulfonimide analog of 11,12-EET vasodilated afferent arterioles of juxtamedullary nephrons. The afferent arteriolar vasodilation elicited by the 11,12-EET analog was significantly attenuated by inhibitors of PKA but was unaltered by inhibition of PKG or guanylyl cyclase. These results demonstrate that activation of PKA is an important intracellular-signaling mechanism responsible for the afferent arteriolar vasodilation elicited by the sulfonimide analog of 11,12-EET.

Acknowledgments

The authors thank Anthony Cook and Sheyla Raibstein for excellent technical assistance with these experiments. This work was supported by grants DK-38226, DK44628 and HL-59699 from the National Institutes of Health and Grant-in-Aid No. 95009790 from the American Heart Association. Dr Edward Inscho is an Established Investigator of the American Heart Association.

References


Afferent Arteriolar Vasodilation to the Sulfonimide Analog of 11,12-Epoxyeicosatrienoic Acid Involves Protein Kinase A

John D. Imig, Edward W. Inscho, Paul C. Deichmann, K. Malla Reddy and John R. Falck

Hypertension. 1999;33:408-413
doi: 10.1161/01.HYP.33.1.408

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1999 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/33/1/408

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/