Evaluating crosstalk between epoxyeicosatrienoic acids (EETs) and neuropeptide signaling in neurogenic vasodilation

Jeffrey J. Iliff1,2 and Nabil J. Alkayed1,2

1Department of Anesthesiology and Peri-Operative Medicine, 2Department of Physiology and Pharmacology
Oregon Health & Science University, Portland OR, USA

Background

- Epoxyeicosatrienoic acids (EETs) are products of cytochrome P450 (CYP) epoxygenase metabolism of arachidonic acid. Cellular EETs levels, release and function are importantly regulated by their metabolism to dihydriperoxidinol ether acids (sEHs), which is catalyzed by the enzyme soluble epoxide hydrolase (sEH).

- sEHs are potent vasodilators in the cerebral circulation produced both in the cerebrovascular endothelium and by cortical astrocytes.

- The cerebrovascular surface vasculature is innervated by extrinsic perivascular nerves, including parasympathetic vasodilator fibers from the sphenopalatine ganglion (SPG) and vasodilator sensory afferents that project to the trigeminal ganglia (TG).

- We have recently reported the expression of EETs-synthetic CYP-2J and sEH-regulatory sEH proteins in the rat SPG and TG, in addition to parasympathetic and trigeminal perivascular fibers innervating the middle cerebral artery (MCA) below.

- Neurogenic cerebral vasodilation is blocked by the putative EETs antagonist, 14,15-EEZE (right). These findings suggest that neurogenic EETs participate in the regulation of cerebral blood flow by perivascular vasodilator nerves.

In this study, we evaluate two mechanistic explanations that account for the expression of EETs-related enzymes in perivascular vasodilator fibers and the functional effect of the EETs antagonist upon neurogenic CBF regulation:

1. Neurogenic EETs act on the cerebral vasculature to modulate the vasomotor action of the parasympathetic and sensory neuropeptides VIP and CGRP.

2. sEHs facilitate the release of the vasactive neuropeptide CGRP from trigeminal neurons.

Methods

- Primary Cerebral Neovascular Culture: Human cerebral neovascular cultured from primary isolated cerebral endothelial cells (CCECs) were grown on a collagen I-coated 96-well plate for 7 days. CCECs were treated with or without the EETs (100 nM, 1000 nM) for 15 min, and then stimulated with or without the neuropeptides (100 nM, 1000 nM) for 15 min.

- Neurogenic CBF Stimulation: CBF was measured using an in vitro mouse calvarial window model. Aortic blood flow was measured using laser Doppler flowmetry before and after CBF stimulation.

- Post-Junctional Modulation of Vasodilator Effects of Neuropeptide by EETs: A rat closed cranial window was utilized to assess the effect of venous vasodilator neuropeptides on the cerebral surface upon CBF, as measured by laser Doppler flowmetry.

Conclusion

In the present study, we report the following findings:

1. Cultured rat trigeminal neurons express both CYP-2J synthase and sEH protein.

These findings confirm that this expression is biochemically necessary for EETs synthesis and regulation.

2. Exogenous EETs do not alter the in vivo cerebrovascular responses to the vasodilatory neuropeptides VIP and CGRP.

These findings suggest that neurogenic EETs do not act post-junctionally to modulate the cerebral vasomotor response to neuropeptides released from extrinsic perivascular nerves.

3. Inhibition of the EETs signaling pathway attenuated stimulus-evoked CGRP release from culture trigeminal neurons.

These findings suggest that neurogenic EETs act intracellularly to mediate neuropeptide release from perivascular neurons.

The regulation of neuropeptide release from trigeminal sensory afferents suggests an important contribution of EETs to the function of extrinsic perivascular vasodilator fibers innervating the cerebral surface vasculature.

Based upon this role, the epoxyeicosatrienoic pathway may play a novel therapeutic target in neurovascular disorders characterized by trigeminovascular dysfunction, such as migraine.

Acknowledgements

The authors thank Dr. Darryl Zebal for the gift of rabbit and human CYP-2J antibody.

Work supported by OHSU Graduate Student Fellowship for the Neurobiology of Disease, a Graduate Student Scholarship from Neuron Pharmaceuticals, NIHES F31NS064949 (J2), and NINDS RO1NS09431 and P01NS054915 (NA).

Funded by the Oregon Brain Institute

Regulation of Neuropeptide Release from Trigeminal Neurons by EETs

Trigeminal neurons were cultured for four days, then stimulated for 60 min with the TRPV1 agonist capsaicin (100 nM) or depolarizing K+ (60 nM). Resulting CGRP release was assayed by ELISA. We tested the hypothesis that inhibition of the EETs signaling pathway would impair CGRP release.

Upper: 30 min pre-treatment with the putative EETs antagonist 15,15-EEZE attenuated basal control (CGRP) release. In addition to capsaicin- and K+-stimulated CGRP release (n = 8)

Lower: 30 min pre-treatment with the P450 sEHs inhibitor MS-PPOH (20 µM) significantly reduced K+-stimulated CGRP release. However, MS-PPOH did not significantly alter capsaicin-evoked CGRP release (n = 3).

CYP-2J and sEH Expression in Trigeminal Neurons

The trigeminal neurons (TNs) were cultured for four days. Immunofluorescent labeling revealed dominant CYP-2J expression in neuronal soma, with less intense labeling extending through dendritic processes. Double labeling revealed that CYP-2J (green) co-localized in TNs with the neuropeptide CGRP (red, n = 3).

Post-Junctional Action

Vasoconstriction

Intracellular Action

Vasodilation

Administration of CGRP to the cerebral surface produced a concentration-dependent increase in cortical blood flow. This effect (EC50 = 6.44 µM) was not altered by 30 min pre-treatment with exogenous 14,15-EET (100 µM, n = 5).

Administration of VIP to the cerebral surface evoked a concentration-dependent vasodilation. This effect (EC50 = 475 nM) was not altered by 30 min pre-treatment with exogenous 14,15-EET (100 µM, n = 5).