Effects of Inhibition of Soluble Epoxide Hydrolase on Microglia Activation

Wang J, Nakayama K, Young J, Campbell C, Koerner IP

Department of Anesthesiology & Peri-Operative Medicine, OHSU, Portland, OR

Background

Soluble epoxide hydrolase (sEH), which inactivates the arachidonic acid metabolite epoxyeicosatrienoic acid (EET), has been linked to ischemic brain injury. Pharmacological inhibition [1] or genetic deletion [2] of sEH reduces infarct size after experimental stroke. This may in part be due to a direct cyto-protective effect of EET and of sEH inhibition on neurons [3]. However, sEH is also a pro-inflammatory enzyme [4], and cerebral inflammation contributes to stroke damage [5]. We therefore tested the hypothesis that EET and inhibition of sEH suppress activation of microglia, thereby reducing the brain's inflammatory response after ischemia.

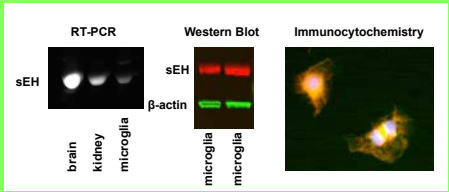
Methods

- primary mixed-glia cultures are generated from cortex of postnatal day 1 Sprague-Dawley rats and grown to confluence in DMEM with 10% fetal bovine serum (FBS)
- on day in-vitro (DIV) 10-14 microglia are harvested by shaking flasks (2 hours, 200 rpm) and re-plated at $5x10^5$ cells/ml
- after 24 hours, medium is replaced with serum-free macrophage medium (Invitrogen)
- 14,15 EET (Cayman Chemical) or the sEH inhibitor 4-phenylchalcone oxide (4-PCO, Biomol) is added 2 hours before stimulation with 100 U/ml controlled standard endotoxin (CSE, Associates of Cape Cod)
- Culture medium is harvested 24 hours after stimulation and cells are fixed with 4% fresh paraformaldehyde for immunocytochemistry or lyzed for protein extraction
- NO release is measured with Griess reagent (Promega)
- TNF alpha (R&D) and PGE_2 (Cayman Chemical) release into the culture medium are measured by ELISA
- NFkB p65 concentration in microglia nuclear extracts is measured by ELISA (Active Motif)
- polyclonal rabbit anti-sEH (Cayman) and anti-NFkB P65 (Santa Cruz), as well as mouse monoclonal anti-Ox42 (Chemicon) and β-actin (Sigma) are used for fluorescent immunocytochemistry and Western Blot
- ANOVA and paired t-test are used to compare groups

[1] Zhang W et al. J Cereb Blood Flow Metab 2007; 27:1931
[2] Zhang W et al. Stroke 2008;39:2073
[3] Koerner IP et al. J Neurosci 2007;27:4642
[4] Schmelzer KR et al. Proc Natl Acad Sci USA 2005;102:9772
[5] Baron FC et al. J Cereb Blood Flow Metab 1999;19:819

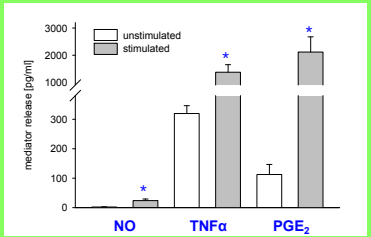
Results

Cortical microglia express soluble epoxide hydrolase



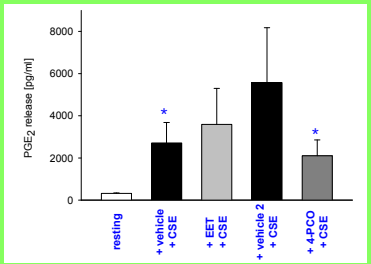
- **sEH mRNA as well as protein are readily detected in primary rat cortical neurons**

Stimulated microglia release NO, TNFα, and PGE_2



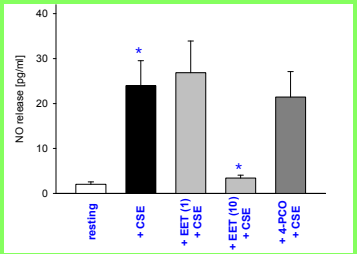
- **unstimulated microglia release only small amounts of inflammatory mediators NO, TNFα and PGE_2**
- **release of all three mediators into the culture medium increases significantly 24 hours after endotoxin stimulation**

Soluble epoxide hydrolase inhibition reduces PGE_2 release



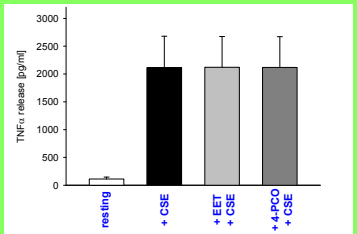
- **release of PGE_2 is reduced in microglia pre-treated with sEH inhibitor 4-PCO (2 μM, 4-PCO +CSE), but not 14,15-EET (1 μM, EET+CSE), before endotoxin (CSE) stimulation**

EET dose-dependently reduces NO release



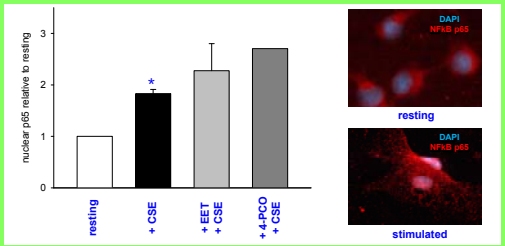
- **pre-treatment with 10 μM (EET 10), but not 1 μM (EET 1), 14,15-EET significantly reduces NO release from stimulated microglia**
- **sEH inhibition (4-PCO) has no effect on NO release**

EET and sEH inhibition do not affect TNFα release



- **TNFα release into the culture medium is increased after endotoxin stimulation (CSE)**
- **EET and sEH inhibition have no effect on TNFα release**

EET and sEH inhibition do not affect NFkB translocation



- **NFkB p65 translocates to the nucleus after endotoxin stimulation**
- **pretreatment with 14,15-EET or 4-PCO does not prevent NFkB activation**

Conclusion

Microglia produce and release the potentially neurotoxic inflammatory mediators nitric oxide (NO), tumor necrosis factor alpha (TNFα), and prostaglandin E_2 (PGE_2) in response to stimulation. Pre-treatment with epoxyeicosatrienoic acid (EET) can decrease NO production in a dose-dependent manner, and PGE_2 release from stimulated microglia can be reduced by inhibition of soluble epoxide hydrolase (sEH). Both effects are independent of NFkB activation, and TNFα translation is not altered. The EET-independent effect of sEH inhibition might potentially be mediated through altered levels of the sEH product DHET, although this has not been tested. Further work is needed to unravel the signaling pathway involved. Reduced release of inflammatory mediators from activated microglia may improve neuronal survival and contribute to the reduction of infarct size achieved by sEH inhibition and EET treatment in experimental stroke. Inhibition of sEH may be a useful therapeutic approach to reduce damage by microglia activation in stroke and neurodegenerative disease.

Supported by Medical Research Foundation of Oregon.

korneri@ohsu.edu