Protective Action of Prostamide E2 on Homocysteine-induced NLRP3 Inflammasome Activation and Podocyte Injury

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ABSTRACT

Recent studies have demonstrated that homocysteine (Hcys)-induced podocyte damage and subsequent podocyte injury and glomerular sclerosis are attributed to the activation of NLRP3 inflammasomes. Given the possible immunosuppressive effect of endogenous cannabinoids, the present study was designed to test whether prostamide E2 (PE2), COX-2 metabolite of anandamide (AEA) diminishes Hcys-induced NLRP3 inflammasome activation and podocyte injury. By confocal microscopy, Hcys treatment of podocytes was found to induce NLRP3 inflammasome formation as demonstrated by the increase in colocalization between three major inflammasome proteins-NLRP3, ASC and caspase-1. Accompanying these effects, elevated Hcys resulted in increased caspase-1 activity and IL-1β production in podocytes. This increase in Hcys-induced NLRP3 inflammasome formation and activation was substantially inhibited by PE2 pretreatment with a maximum inhibition >60% of the Hcys-induced caspase-1 activity and IL-1β production. Anandamide only had such inhibitory effect on inflammasome activation at a concentration 10 times higher than PE2, which was blockable by cox-2 inhibitor. Furthermore, PE2 was found to preserve podocyte morphology by maintaining the distinct arrangement of F-actin fibers normally lost in response to Hcys. It also prevented podocyte dysfunction by restoring Hcys-induced suppression of VEGF production and secretion and by inhibition of Hcys-induced decreases in podocin and increases in desmin. Together, these results provide evidence that PE2 may exert its anti-inflammatory action by suppression of Hcys-induced NLRP3 inflammasome activation and prevention of podocyte injury and dysfunction (supported by NIH grants HL057244, HL-75316 and DK54927).

BACKGROUND

- Our previous studies have demonstrated that Hcys-induced NLRP3 inflammasomes activation is one of major initial mechanism resulting in podocyte injury.
- Cyclooxygenase-2 (COX-2) and its major product Prostaglandin E2 (PGE2) have been considered as important mediator to maintain the podocyte and glomerular function. However, it remains unknown whether COX-2 products from other substrates such as anandamide (AEA) are also important in the regulation of podocyte and glomerular function.
- Our recent studies have shown that an AEA product via COX-1, prostamide E2, regulates renal medullary blood flow and sodium excretion exerting an action as a natriuretic factor.

The present study was designed to test whether protasmide E2 (PE2) protects podocytes from Hcys-induced inflammasome activation and associated injury.

METHODS

Cell culture. Conditionally immortalized mouse podocytes (kindly provided by Dr. Paul Klotman, Division of Nephrology, Department of Medicine, Mount Sinai School of Medicine, New York, NY, USA) were cultured at 33°C on collagen I-coated flasks or plates in RPMI 1640 medium supplemented with recombinant mouse interferon–γ. The cells were then differ/entiated by culturing at 37°C for 10-14 days in medium without interferon–γ prior to their use in experiments**.**

Immunofluorescent staining. Cells were grown on coverslips and fixed in PBS containing 4% paraformaldehyde for 10 minutes. Donkey serum (5%) (Sigma, St. Louis, MO) was included in all blocking and primary and secondary antibody buffers. Coverslips were incubated with primary antibodies overnight at 4°C. Secondary antibodies were Alexa Fluor-conjugated (Invitrogen, Inc., Grand Island, NY). Coverslips were mounted in Vectashield reagent containing DAPI (Vector Laboratories, Inc., Burlingame, CA). The fluorescent images were obtained using a confocal laser scanning microscope (Fluoview 1000, Olympus, Japan) and analyzed using Image J software.

Caspase-1 activity, IL-1β and VEGF production. Cell lysate was collected for caspase-1 activity assay (Caspase-1 Colorimetric Assay Kit, BioVision), and cultured medium was collected for IL-1β and VEGF production assay by using commercially available kits (Bender Medsystems; R&D Systems).

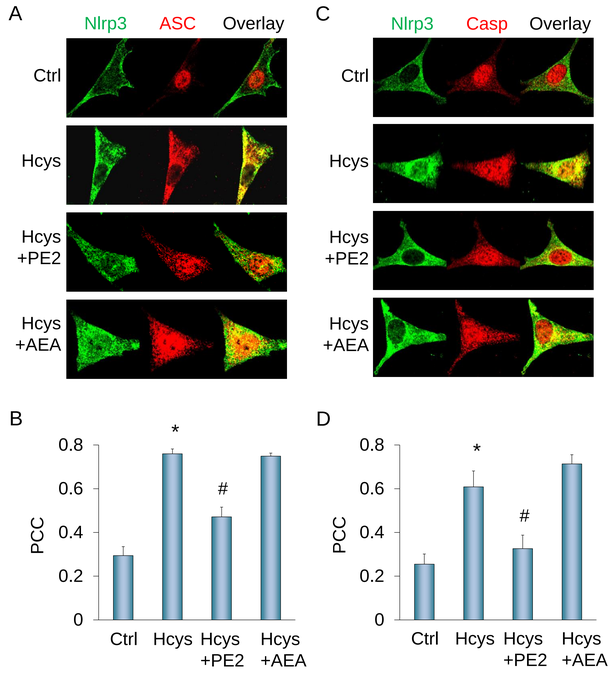

Figure 1. Effects of PE2 and AEA on Hcys-induced inflammasomes activation. (A) Representative confocal fluorescent images in podocytes after stimulation of Hcys or with PE2 (10 μM, pre-treat 30mins) or AEA (10 μM, pre-treat 30mins). (B) Summarized co-localization coefficient data showing that co-localization levels of NALP3 with ASC or caspase-1 were attenuated by PE2 but not by AEA. n=6 batches of cells, * P<0.05 vs. Ctrl group; # P<0.05 *vs.* corresponded Hcys group.

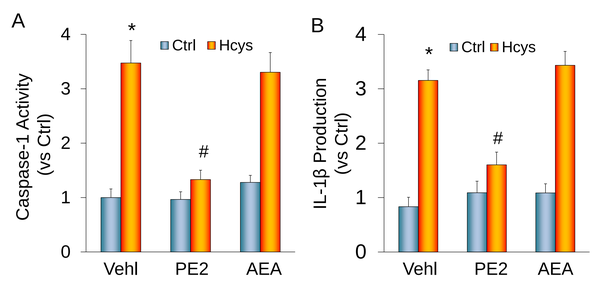

Figure 2. Effects of PE2 and AEA on Hcys-induced augmentation of caspase-1 activity and IL-1β production. (A) Caspase-1 activity measurement and (B) IL-1β production measurement in podocytes with Hcys, Hcys+PE2, Hcys+AEA. It was shown that PE2 but not AEA attenuated Hcys-induced increases in caspase-1 activity and IL-1β production. n=6 batches of cells, * P<0.05 *vs.* Ctrl group; # P<0.05 *vs.* Vehl-Hcys group.

RESULTS

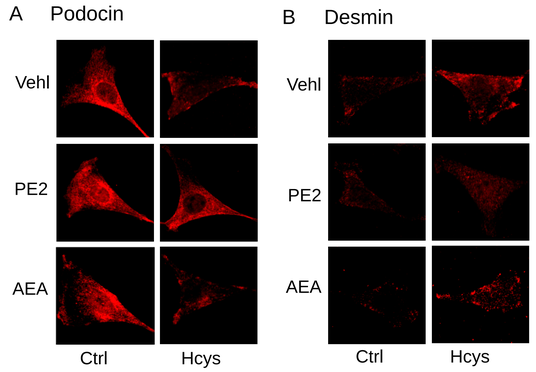

Figure 3. Pretreatment of PE2 preserved podocyte function as observed by fluorescence microscopy of podocin as normal podocyte marker and desmin as podocyte injury marker. PE2 but not AEA blocked Hcys-induced decrease in podocin levels in podocytes (A) and prevented Hcys-stimulated increase in desmin (B). n=6.

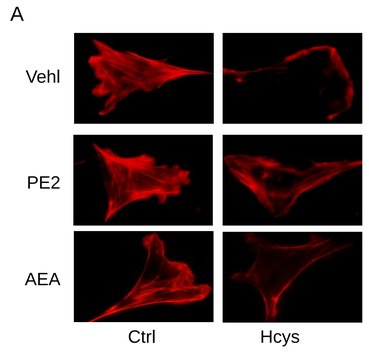

Figure 4. Representative microscopic images showing F-actin expression and arrangement in podocytes using rhodamine-phalloidin staining (A). F-actin was found to be aggregated around the periphery of cells after Hcys stimulation. However, the pattern was partially restored by PE2 pre-treatment but not by AEA. In addition, Hcys was able to compromise podocyte function, evident by decreased VEGF secretion, which was restored by PE2 but not by AEA (B). n=6. *p<0.05 vs. Ctrl group, #p<0.05 vs. Vehl-Hcys group.

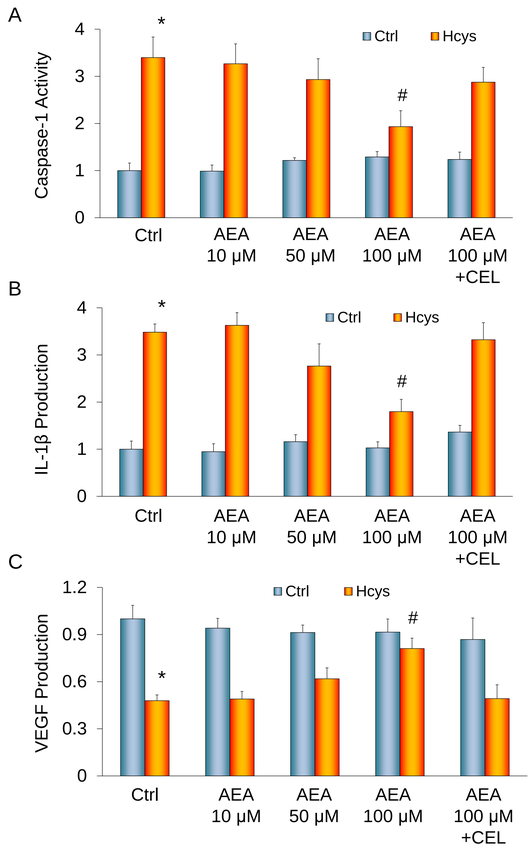

Figure 5. Effects of AEA in high concentrations on Hcys-induced NLRP3 inflammasome activation and podocyte injury. High dose of AEA (100 μM), but not low dose was able to significantly inhibit the NLRP3 inflammasome activation as shown by the decrease in caspase-1 activity (A) and IL-1β production (B). In addition, the pretreatment of selective Cox-2 inhibitor, celexocib (CEL), significantly blocked the effect of high dose of AEA. By VEGF assay, Hcys-induced podocyte dysfunction was also recovered by high dose of AEA, which was inhibited by CEL (a COX-2 inhibitor) pretreatment. n=6. *p<0.05 vs. Ctrl group, #p<0.05 vs. Vehl-Hcys group.

CONCLUSION

Our results provide evidence to support the view that PE2 suppresses Hcys-induced NLRP3 inflammasome activation in podocytes and thereby prevents these cells from Hcys-induced injury and dysfunction.