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Soluble epoxide hydrolase in podocytes is a significant contributor to renal function under hyperglycemia

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ABSTRACT

Background: Diabetic nephropathy (DN) is the leading cause of renal failure, and podocyte dysfunction contributes to the pathogenesis of DN. Soluble epoxide hydrolase (sEH, encoded by *Ephx2*) is a conserved cytosolic enzyme whose inhibition has beneficial effects on renal function. The aim of this study is to investigate the contribution of sEH in podocytes to hyperglycemia-induced renal injury.

Materials and methods: Mice with podocyte-specific sEH disruption (pod-sEHKO) were generated, and alterations in kidney function were determined under normoglycemia, and high-fat diet (HFD)- and streptozotocin (STZ)-induced hyperglycemia.

Results: sEH protein expression increased in murine kidneys under HFD- and STZ-induced hyperglycemia. sEH deficiency in podocytes preserved renal function and glucose control and mitigated hyperglycemia-induced renal injury. Also, podocyte sEH deficiency was associated with attenuated hyperglycemia-induced renal endoplasmic reticulum (ER) stress, inflammation and fibrosis, and enhanced autophagy. Moreover, these effects were recapitulated in immortalized murine podocytes treated with a selective sEH pharmacological inhibitor. Furthermore, pharmacological-induced elevation of ER stress or attenuation of autophagy in immortalized podocytes mitigated the protective effects of sEH inhibition.

Conclusions: These findings establish sEH in podocytes as a significant contributor to renal function under hyperglycemia.

General significance: These data suggest that sEH is a potential therapeutic target for podocytopathies.

1. Introduction

The incidence of type 2 diabetes mellitus continues to grow in the United States and worldwide, paralleling the obesity epidemic [1]. Diabetic nephropathy (DN) is a devastating complication of diabetes and the leading cause of end-stage renal disease (ESRD) [2]. DN accounts for about 40% of new cases of ESRD, and approximately 44% of new dialysis patients in the United States are diabetics [3,4]. Clinical hallmarks of DN include persistent albuminuria and increased creatinine clearance as a result of a decline in the glomerular filtration rate and alterations in the glomerular basement membrane (GBM). Podocytes are significant contributors to the integrity of the GBM, and growing evidence implicates podocyte dysfunction in the pathogenesis of DN [5,6]. Given the role of podocytes in normal renal function and injury, elucidating the molecular mechanisms underlying podocyte function is critical for understanding DN pathogenesis and developing effective therapies.

Arachidonic acids and other polyunsaturated fatty acids are

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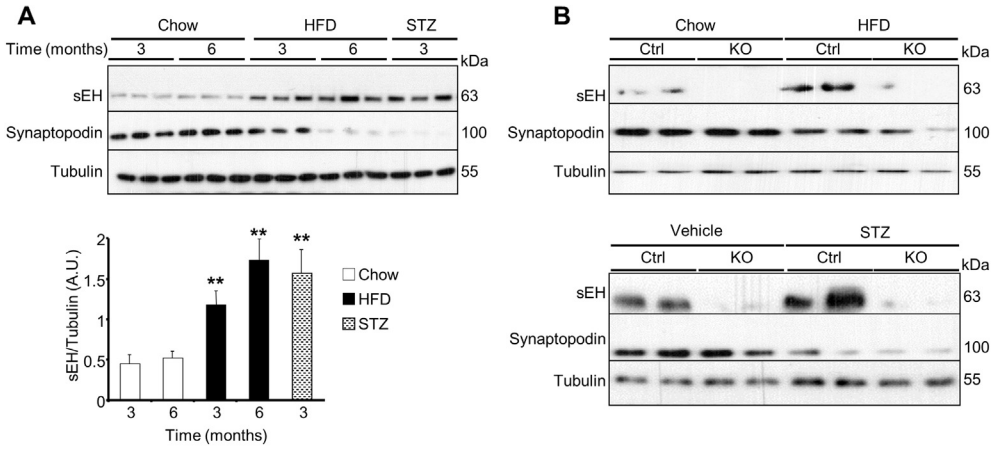

Fig. 1. Increased renal sEH expression upon HFD and STZ challenges. A) Immunoblots of sEH and synaptopodin expression in total kidney lysates of wild-type male mice fed standard laboratory chow and HFD (for 3 and 6 months) and those administered STZ (3 months). Each lane represents lysate from a different animal, and representative immunoblots are shown. Bar graph represents sEH normalized to tubulin expression and presented as means ± SEM from three independent experiments. $^{**}p \leq 0.01$ indicates a significant difference between HFD or STZ-treated mice and age-matched controls. B) Isolated glomeruli from control (Ctrl; n = 6) and pod-sEHKO (KO; n = 6) mice fed standard laboratory chow and HFD for 3 months (top panel) and STZ-treated (bottom panel) were immunoblotted for sEH, synaptopodin, and tubulin. Representative immunoblots are shown. A.U.: arbitrary unit.

metabolized by cyclooxygenases, lipoxygenases, and cytochrome P450s (CYP) to eicosanoids and related oxylipins which are key regulators of numerous biological processes. CYP epoxygenase enzymes (including CYP2C, 2J) metabolize arachidonic acid to biologically active epoxyeicosatrienoic acids (EETs) [7] which are anti-hypertensive and anti-inflammatory [8,9]. However, EETs are rapidly hydrolyzed to a large extent by the soluble epoxide hydrolase (sEH, encoded by *Ephx2*) into the less biologically active metabolites, dihydroxyeicostrienoic acids (DHETs) [10]. sEH is a cytosolic enzyme that is widely distributed and highly expressed in the kidney and liver [11]. Several studies highlight the role of sEH in renal disease and the therapeutic potential of inhibiting this enzyme to increase EETs concentrations. Indeed, pharmacological inhibition of sEH reduces renal injury and inflammation in a salt-sensitive hypertension model [12]. Also, sEH inhibition prevents renal interstitial fibrosis [13], and *Ephx2* whole-body deficient mice display reduced renal inflammation and injury [12,14]. While these studies implicate sEH in renal function, they utilize systemic approaches and the contribution of sEH in podocytes to DN remains undetermined. Recently, we report that podocyte sEH deficiency attenuates lipopolysaccharide-induced kidney injury [15]. In the current study, we investigated the effects of podocyte-specific sEH deficiency on renal function under normoglycemia and hyperglycemia and determined the underlying molecular mechanism.

2. Materials and methods

2.1. Mouse studies

Mice with podocyte-specific sEH disruption (pod-sEHKO) were generated as we recently described [15]. Briefly, sEH floxed (*Ephx2*$^{fl/fl}$) mice were bred to transgenic mice expressing Cre recombinase under control of the podocin promoter. Mice were maintained on a 12-hour light-dark cycle with free access to food and water and were fed standard laboratory chow (Purina lab chow, # 5001) or a high-fat diet (HFD, 60% kcal from fat, # D12492, Research Diets). For streptozotocin (STZ)-induced hyperglycemia 8–12 week old pod-sEHKO (*Ephx2*$^{fl/fl}$ Pod-cre^{+}) and control (*Ephx2*$^{fl/fl}$) male mice received a single intraperitoneal injection of STZ (160 µg/g body weight) as detailed in Supplemental Information. Metabolic parameters were determined in serum and urine from fed and fasted mice as described in Supplemental Information. Mice were sacrificed at 6 and 3 months after STZ and HFD challenges, respectively. Tissues were collected at the time of sacrifice for biochemical and histological analyses. Kidneys were ground in liquid nitrogen, lysed using RIPA buffer then used for Western blotting. Also, kidney sections were fixed and transmission electron microscopy performed as detailed in Supplemental Information. All mouse studies were conducted in line with federal regulations and were approved by the Institutional Animal Care and Use Committee at University of California Davis.

2.2. Cell culture and glomerular isolation

E11 murine podocyte cell line was cultured and differentiated as described in Supplemental Information. Glomeruli were isolated from pod-sEHKO (*Ephx2*$^{fl/fl}$ Pod-cre^{+}) and control (*Ephx2*$^{fl/fl}$) mice as detailed in Supplemental Information.

2.3. Statistical analyses

Data are expressed as means + standard error of the mean (SEM). Statistical analyses were performed using JMP program (SAS Institute). Post-hoc analysis was performed using Tukey-Kramer honestly significant difference test. The number of animals used is indicated in the figure legends. For biochemistry studies, comparisons between groups were performed using unpaired two-tailed Student's *t*-test. The log-rank test was used to compare survival curves. Differences were considered significant at $p \leq 0.05$ and highly significant at $p \leq 0.01$.

3. Results

3.1. Hyperglycemia increases renal sEH expression

We determined renal sEH expression in wild-type mice under normoglycemia and HFD- and STZ-induced hyperglycemia. Immunoblots of kidney lysates revealed increased sEH protein expression under hyperglycemia concomitant with decreased synaptopodin expression as previously reported [16,17] (Fig. 1A). Similarly, immunoblots of isolated glomeruli from wild-type mice demonstrated increased sEH protein expression under HFD- and STZ-induced hyperglycemia compared with normoglycemia (Fig. 1B). Notably, isolated glomeruli from pod-sEHKO mice did not exhibit significant sEH expression demonstrating the efficiency of deletion and high selectivity of sEH antibodies. Together, these data establish increased renal sEH protein expression under hyperglycemia and suggest that sEH may impact hyperglycemia-induced renal injury.

3.2. Podocyte-specific sEH deficiency improves renal function under hyperglycemia

To investigate the contribution of sEH in podocytes to hyperglycemia-induced renal injury, we generated mice with podocyte-specific sEH disruption. Pod-sEHKO mice exhibit specific and efficient genetic disruption of sEH in podocytes as we previously described [15]. The effects of podocyte sEH disruption on renal function under