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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.

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Conflict of interest: AB, BI, BDH, DCZ and FGH are co-inventors on a patent on the use of soluble epoxide hydrolase inhibitors to treat diabetic nephropathy. BI and BDH are co-founders of EicOsis LLC, to move sEH inhibitors to the clinic to treat neuropathic and inflammatory pain.

Authors Contribution:
Design: Bettaieb and Haj
Conduct/data collection: Bettaieb, Shinichiro, Hsu, Ito, Chahed, Bachaalany, Gruzdev, Calvo-Rubio, and Inceoglu
Analysis: Bettaieb, Shinichiro, Calvo-Rubio, Villalba, Imig, Zeldin, Hammock and Haj
Contributed reagents: Lee, Gruzdev, Zeldin, and Hammock
Writing manuscript: All authors were involved in writing and editing the manuscript and had final approval of the submitted and published versions

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.

Keywords

Diabetic nephropathy; soluble epoxide hydrolase; podocyte; knockout mice; autophagy; endoplasmic reticulum stress

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 metabolized by cyclooxygenases, lipoxygenases, and cytochrome P450's (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.

Materials and Methods

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 24 and 13 weeks 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.

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.

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 $p \leq 0.01$.

Results

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.

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 normoglycemia and hyperglycemia were investigated as detailed in methods. Chow-fed control and knockout mice exhibited similar body weights while STZ treatment led to a comparable decrease in body weights of both groups (Fig. 2A). Also, kidney weights increased in STZ-treated control mice but to a lesser extent in pod-sEHKO (Fig. 2B, C). A monitor for renal injury is albuminuria which is an early marker of renal damage in many kidney diseases [18, 19]. Urine albumin/creatinine (ACR) increased upon STZ challenge, but that was significantly less in pod-sEHKO mice compared with controls indicative of preserved renal function (Fig. 2D). Also, blood urea nitrogen (BUN) level was measured to evaluate the ability of the kidney to remove urea from blood (elevated BUN correlate with decreased renal function[20]). STZ-induced hyperglycemia led to a significant increase in BUN, but that was significantly less in pod-sEHKO mice compared with controls consistent with preserved renal function in the former (Fig. 2E). Moreover, STZ challenge increased fasted and fed serum glucose concentrations, but these were significantly less in pod-sEHKO mice compared with controls suggestive of better glucose control (Fig. 2F, G). Comparable preservation of renal function and glucose control in pod-sEHKO mice was observed in an independent cohort of STZ-treated mice (data not shown). Furthermore, and consistent with the beneficial effects of podocyte sEH deficiency, pod-sEHKO mice survived longer than controls under STZ-induced hyperglycemia (Fig. 2H). Also, under HFD-induced hyperglycemia pod-sEHKO mice exhibited better glucose control compared with control mice but without significant changes in ACR or BUN, likely due to the relatively short duration of HFD feeding (Table S1). Collectively, these data establish protective effects of podocyte sEH deficiency on renal function under hyperglycemia.

Podocyte sEH deficiency attenuates hyperglycemia-induced renal injury

Systemic sEH deficiency and pharmacological inhibition reduce renal injury [12, 14]. The effects of podocyte-specific sEH deficiency on the hyperglycemia-induced renal injury were

determined using Periodic Acid-Schiff stain (PAS), toluidine blue stain and electron microscopy. After 6 months of STZ challenge, significant widening of the interstitial space and thickened basement membranes were observed in control mice while changes to the glomerular structure were significantly less in knockouts (Fig. S1A). Additionally, while no obvious differences were noted basally in toluidine blue-stained sections, hyperglycemia caused severe damage to the kidneys of control mice but to a lesser extent in knockouts as evidenced by distorted architecture of the glomeruli and tubules, flattened epithelia and nuclear and epithelial debris in the lumina (black arrow in Fig. S1B). Moreover, electron microscopy revealed significant alterations in the morphology of podocytes in STZ-treated mice (Fig. S1C). Swollen podocytes with large cytoplasmic vacuoles and effaced foot processes (black arrow) were observed in STZ-treated control mice. In contrast, pod-sEHKO mice exhibited only mild focal foot process effacement indicative of lower STZ-induced renal damage (white arrow). These observations indicate that podocyte-specific sEH deficiency protects podocyte structure and foot processes against hyperglycemia-induced damage.

Podocyte sEH deficiency mitigates hyperglycemia-induced endoplasmic reticulum stress and inflammation

Endoplasmic reticulum (ER) stress is implicated in the pathogenesis of DN [21], and sEH deficiency and pharmacological inhibition attenuate ER stress [22, 23]. Accordingly, the effects of podocyte sEH deficiency on renal ER stress were determined in control and pod-sEHKO mice under normoglycemia and STZ- and HFD-induced hyperglycemia. We evaluated activation of ER transmembrane sensors inositol-requiring enzyme 1α (IRE1α) and PKR-like ER-regulated kinase (PERK) and its downstream target α-subunit of eukaryotic translation initiation factor 2 (eIF2α) [24, 25]. STZ induced renal ER stress as evidenced by increased PERK (Thr^{980}), eIF2α (Ser^{51}) and IRE1α (Ser^{724}) phosphorylation (Fig. 3A). Importantly, pod-sEHKO mice exhibited significant attenuation of STZ-induced ER stress compared with controls as evidenced by decreased PERK, eIF2α, and IRE1α phosphorylation. sEH deficiency and pharmacological inhibition exhibit anti-inflammatory effects partly through attenuating NF-κB inflammatory response [26], so renal NF-κB signaling was determined in control and pod-sEHKO mice under normoglycemia and hyperglycemia. STZ induced a renal inflammatory response as evidenced by increased IKKα ($Ser^{178/180}$), IκB (Ser^{32}) and NF-κBp65 (Ser^{536}) phosphorylation and NF-κBp50 expression (Fig. 3B). Hyperglycemia-induced IKKα, IκBα and NF-κBp65 phosphorylation and NF-κBp50 expression were significantly attenuated in pod-sEHKO mice compared with controls. Notably, comparable attenuation of ER stress and inflammation was also observed in pod-sEHKO mice compared with controls under HFD-induced hyperglycemia (Fig. S2A, B). Altogether, these findings demonstrate that podocyte sEH deficiency is associated with decreased hyperglycemia-induced renal ER stress and inflammation.

Podocyte sEH deficiency enhances autophagy and attenuates fibrosis under hyperglycemia

Autophagy is a well-coordinated fundamental cell process that delivers intracellular constituents to lysosomes for degradation to maintain homeostasis and is a protective mechanism against podocyte injury [27, 28]. The effects of sEH podocyte deficiency on

autophagy were determined in control and pod-sEHKO mice by evaluating proteins that are involved in the formation and maturation of autophagosomes namely Beclin, autophagy-related genes (Atg) and microtubule-associated protein 1A/1B-light chain 3 (LC3-I) and its phosphatidylethanolamine-conjugated form (LC3-II) [29, 30]. Under STZ-induced hyperglycemia, pod-sEHKO mice exhibited enhanced autophagy compared with controls as evidenced by increased Beclin1, LC3-II, and Atg5 and Atg7 expression (Fig. 4A). Also, mRNA of *beclin* and *Lc3* were similarly increased in pod-sEHKO mice compared with controls (Fig. S3). Similarly, pod-sEHKO mice exhibited enhanced autophagy compared with controls under HFD-induced hyperglycemia (Fig. S4A). In line with enhanced autophagy, pod-sEHKO mice exhibited lower STZ-induced fibrosis compared with controls as evidenced by decreased transforming growth factor beta receptor II (TGFβRII) expression and Smad2 phosphorylation [31, 32] (Fig. 4B). On the other hand, no significant differences in TGFβRII expression and Smad2 phosphorylation were observed between pod-sEHKO and control mice under HFD-induced hyperglycemia (Fig. S4B), as features of kidney fibrosis likely require longer feeding duration [33, 34]. Collectively, these data demonstrate that podocyte sEH deficiency is associated with enhanced autophagy and attenuated fibrosis under hyperglycemia.

Pharmacological inhibition of sEH in immortalized podocytes recapitulates the effects of sEH deficiency *in vivo*

The integrated renal response to hyperglycemia incorporates many cell types including podocytes. To decipher the molecular mechanism mediating sEH function in podocytes under hyperglycemia we used E11 murine podocyte cell line. Differentiated E11 podocytes were cultured under normal and high glucose as detailed in methods, and the effects of a selective sEH pharmacological inhibitor (TPPU) were evaluated. Consistent with *in vivo* findings from kidney lysates, high glucose culture of E11 podocytes increased ER stress (IRE1α phosphorylation), inflammation (NF-κBp65 phosphorylation), autophagy (Beclin, LC3-II, Atg5 and Atg7 expression), fibrosis (TGFβRII expression and Smad2 phosphorylation) and cleaved Caspase3 (Fig. 5A). Importantly, sEH pharmacological inhibition mitigated the deleterious effects of high glucose culture with sEH inhibitor-treated podocytes exhibiting decreased ER stress, inflammation, and fibrosis and enhanced autophagy (Fig. 5A). To further delineate the contribution of ER stress and autophagy to the beneficial effects of podocyte sEH deficiency, pharmacological modulation of these pathways was performed as detailed in m ethods. Notably, treatment of E11 podocytes with the chemical chaperone 4-phenylbutyrate (4PBA; Fig. 5B, lane4) to alleviate ER stress or autophagy inducer N-(3-Methylphenyl)-4-(4-pyridinyl)-2-thiazolamine (STF-62247; Fig. 5B, lane5) qualitatively recapitulated the beneficial effects of sEH inhibition. On the other hand, the beneficial effects of sEH pharmacological inhibition under high glucose were significantly mitigated by inducing ER stress using palmitate (Fig. 5B, lane7) or attenuating autophagy using N2,N4-dibenzylquinazoline-2,4-diamine (DBeQ; Fig. 5B, lane8). Altogether, these findings demonstrate that the beneficial effects of sEH podocyte deficiency under high glucose are mediated, at least in large part, through ER stress and autophagy.

Discussion

DN is a devastating complication of diabetes and podocyte dysfunction is a significant contributor to disease pathogenesis. Thus, elucidating the molecular mechanisms underlying podocyte function will aid in developing effective therapies. In the current study, we investigated the role of sEH in podocytes under hyperglycemia using genetic and pharmacological approaches. We report increased sEH expression in glomeruli under HFD- and STZ-induced hyperglycemia. Notably, podocyte-specific sEH ablation preserved renal function and glucose control and mitigated hyperglycemia-induced renal injury. The beneficial effects of podocyte sEH deficiency were associated with and likely caused by decreased ER stress and enhanced autophagy with a corresponding attenuation in inflammation and fibrosis. Moreover, pharmacological modulation of ER stress and autophagy in immortalized podocytes mitigated the beneficial effects of sEH inhibition. Collectively, these findings identify sEH in podocytes as a contributor to renal function under hyperglycemia and suggest that sEH pharmacological inhibition may be of therapeutic value in hyperglycemia-induced renal injury.

Genetic and pharmacological studies implicate epoxygenase metabolites in renal function and suggest that increasing their concentrations may serve as a therapeutic strategy for preventing end-organ damage [35, 36]. sEH genetic deficiency or pharmacological inhibition stabilize EETs and other epoxy fatty acids by preventing their conversion to DHETs or corresponding diols [26]. Therefore, alteration in sEH expression and activity will likely impact renal function. In this regard, we report increased sEH protein expression in mouse glomeruli upon HFD- and STZ-induced hyperglycemia. This finding is consistent with sEH upregulation in podocytes upon lipopolysaccharide challenge [15] and in keeping with observations that enhanced sEH expression is often associated with inflammatory states [9, 37]. On the other hand, some studies using diabetes-associated renal injury rodent models report attenuation [38] or no significant change [39] in renal sEH expression. Reasons for the discrepancy remain to be determined but could be due to differences in the nature and duration of the challenge. Also, it remains to be established if hyperglycemia-induced upregulation of sEH contributes to renal injury and if it is due to decreased EETs and/or elevated diol concentrations. Nevertheless, the beneficial effects of podocyte sEH deficiency suggest that sEH inhibition may have therapeutic implications for hyperglycemia-induced renal injury and DN. It is worth noting that podocyte sEH deficiency partially preserved renal function under hyperglycemia. Indeed, it is likely that additional contributing factors modulate podocyte dysfunction during DN given the complex molecular mechanisms underlying disease pathogenesis (reviewed in [40, 41]).

Podocyte sEH deficiency mitigated the effects of high glucose *in vivo* and culture. A limitation of the study is the use of high-dose STZ to induce hyperglycemia since it may cause toxic effects in the kidney in addition to the intended toxicity to pancreatic β-cells [42]. However, the protective effects of sEH deficiency were also observed, albeit less robustly, in a HFD-induced hyperglycemia model demonstrating that they were not unique to a particular challenge. These findings are in keeping with the renal protective effects of whole-body *Ephx2* deficiency and pharmacological inhibition [12–14], and of podocyte sEH deficiency in attenuating lipopolysaccharide-induced kidney injury [15]. We established that

under hyperglycemia, podocyte sEH deficiency preserved renal function and glucose control. Pod-sEHKO mice exhibited improved renal function compared with controls as evidenced by decreased hyperglycemia-induced albuminuria and BUN concentrations. Consistent with these findings electron microscopy revealed less histopathological features (glomerular hypertrophy and thickening of the GBM [43]) that are associated with albuminuria indicative of lower hyperglycemia-induced renal damage in podocyte sEH deficient mice. Also, pod-sEHKO mice exhibited better glucose control as evidenced by the fed and fasted glucose concentrations. While the underlying mechanism remains to be elucidated several factors may contribute to this observation. (1) Podocyte sEH deficiency may impact another renal cell(s) to enhance glucose clearance and/or decrease renal gluconeogenesis. In healthy individuals, the kidneys contribute 20%–25% of glucose via gluconeogenesis [44]. However, in patients with T2D hepatic and renal gluconeogenesis is increased, but the increase in renal gluconeogenesis is significantly greater than hepatic gluconeogenesis (300% vs. 30%) [45]. (2) We cannot rule out direct or indirect effects of podocyte-specific sEH deletion on other tissue(s) that are implicated in glucose control.

The current study identified signaling nodes that are implicated in mediating sEH action in podocytes under hyperglycemia. Podocyte sEH deficiency *in vivo* and culture was associated with decreased ER stress and enhanced autophagy with a corresponding reduction in NF-κB inflammatory response and fibrosis. Attenuation of hyperglycemia-induced ER stress in sEH-deficient podocytes is consistent with sEH acting as a significant modulator of ER stress [22, 23]. In line with decreased ER stress, podocyte sEH deficiency attenuated hyperglycemia-induced NF-κB inflammatory response. ER stress is implicated in the pathogenesis of DN [21], and chemical chaperones that mitigate ER stress slow DN progression [46, 47]. Hence, it is reasonable to stipulate that the renal protective effects of podocyte sEH deficiency under hyperglycemia are mediated, at least partly, by decreased ER stress. In addition, podocyte sEH deficiency was associated with enhanced autophagy as evidenced by several markers (Beclin, LC3-I/II, and Atg5/7) that are significant contributors to the formation and maturation of autophagosomes. This finding is consistent with enhanced autophagy upon sEH deficiency in other tissues [48, 49]. Podocytes exhibit a high level of autophagy which may serve as a mechanism to maintain cellular homeostasis [50, 51]. Accumulating evidence implicates autophagy in regulating critical aspects of normal and diabetic kidney [27, 28], but its role in DN is somewhat controversial and may depend, at least partly, on the stage of the disease. Indeed, autophagy is inhibited in kidneys of STZ-induced diabetic rodents [52–54], and impaired autophagy is observed in kidney samples of T2D patients [55]. On the other hand, enhanced autophagy is observed in diabetic mice [56] and proximal tubule epithelial cells cultured in high glucose [57]. While additional studies are needed to delineate regulation of autophagy by sEH and its contribution to DN, it is likely that the enhanced autophagy upon sEH podocyte deficiency *in vivo* or pharmacological inhibition in culture constituted a protective mechanism against hyperglycemia-induced podocyte injury. Of note, sEH pharmacological inhibition in immortalized podocytes recapitulated the beneficial effects of sEH deficiency *in vivo* and led to decreased ER stress and enhanced autophagy, consistent with cell-autonomous effect. Importantly, pharmacological induction of ER stress or inhibition of autophagy mitigated the protective effects of sEH inhibition demonstrating cause-effect relation. Altogether, these

findings identify ER stress and autophagy as key mechanisms that mediate the protective effects of podocyte sEH deficiency under hyperglycemia.

In summary, the current study identifies podocytes as significant contributors to the renal protective effects of sEH deficiency under hyperglycemia and suggests that sEH pharmacological inhibition may constitute a therapeutic approach in DN and hyperglycemia-induced podocyte injury.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

1. Podocyte-specific sEH deficiency in mice improved under STZ/HFD-induced hyperglycemia.
2. These effects were associated with decreased ER stress and enhanced autophagy.
3. sEH pharmacological inhibition *in vivo* mitigated hyperglycemia-induced renal injury.

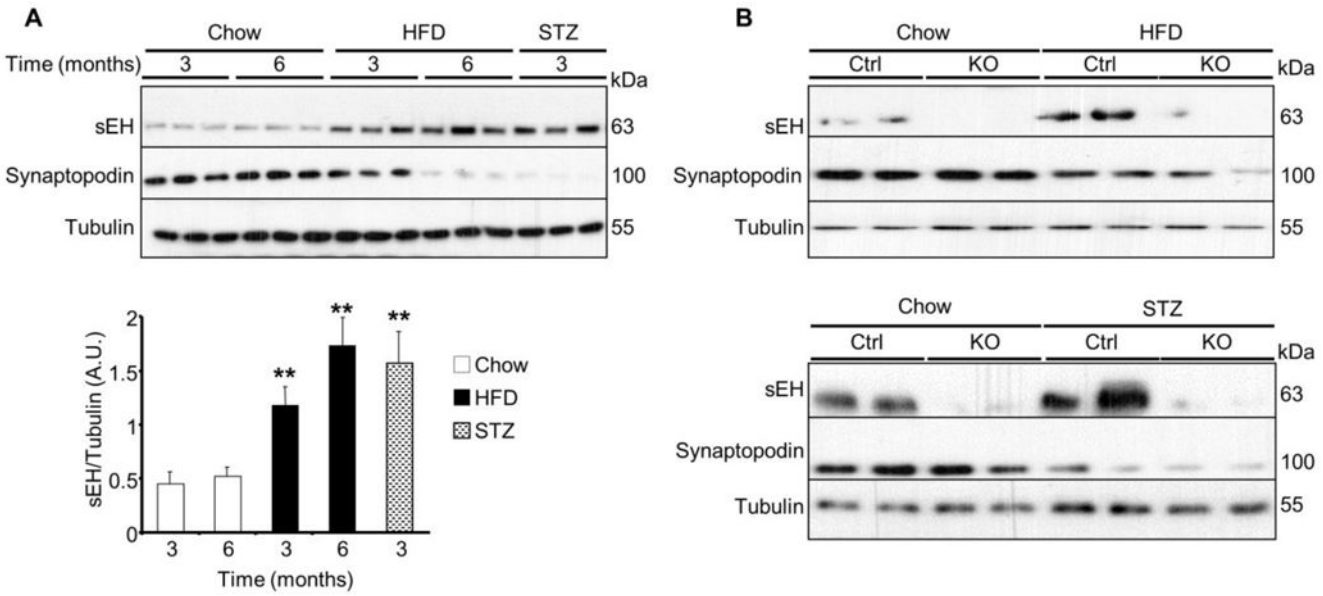

Figure 1. Increased renal sEH expression upon HFD and STZ challenges

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.

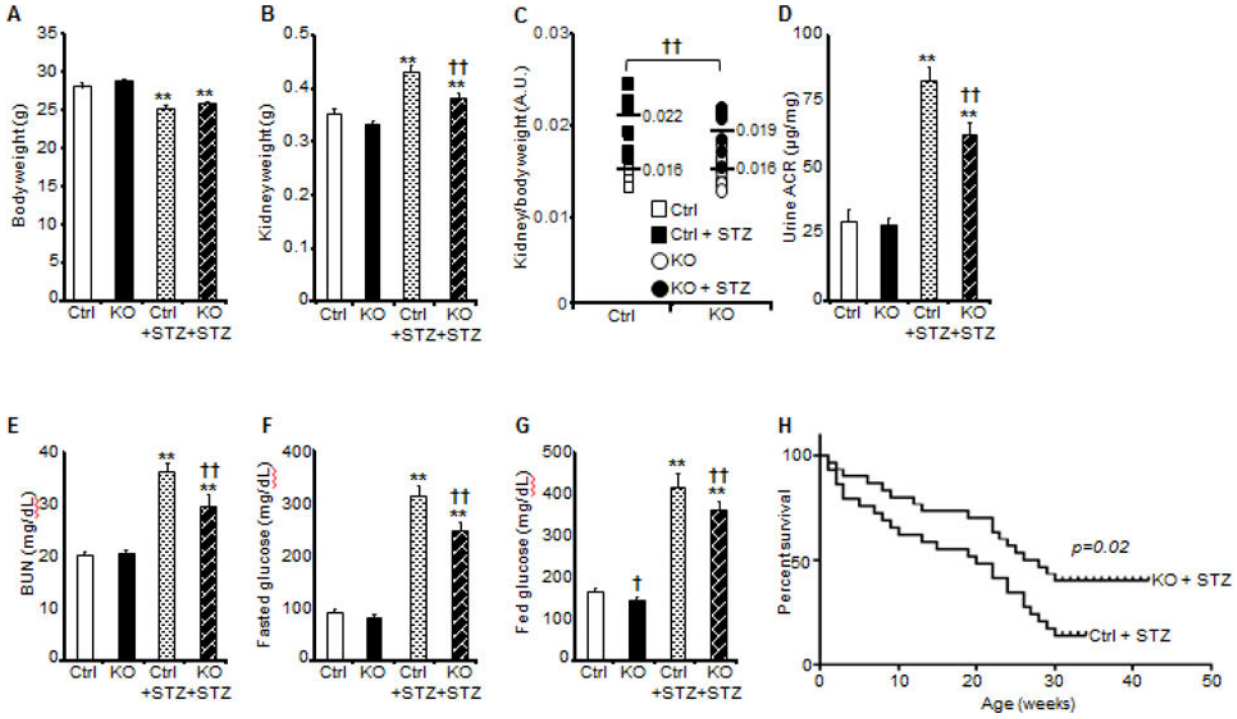

Figure 2. Podocyte-specific sEH deficiency alleviates hyperglycemia-induced changes to renal function

Body (**A**) and kidney (**B**) weights, and kidney to body weight ratio (**C**), urinary albumin to creatinine ratio (**D**), blood urea nitrogen (**E**), fasted (**F**) and fed (**G**) serum glucose concentrations from control (Ctrl; n=13) and pod-sEHKO (KO; n=14) mice without and with STZ at 24 weeks after injection. ** $p<0.01$ indicates a significant difference between vehicle vs. STZ, and † $p<0.05$; †† $p<0.01$ indicate a significant difference between Ctrl vs. KO. **H**) Kaplan-Meier survival curve of STZ-treated Ctrl and KO mice ($p=0.02$, using log-rank test). A.U: arbitrary unit.

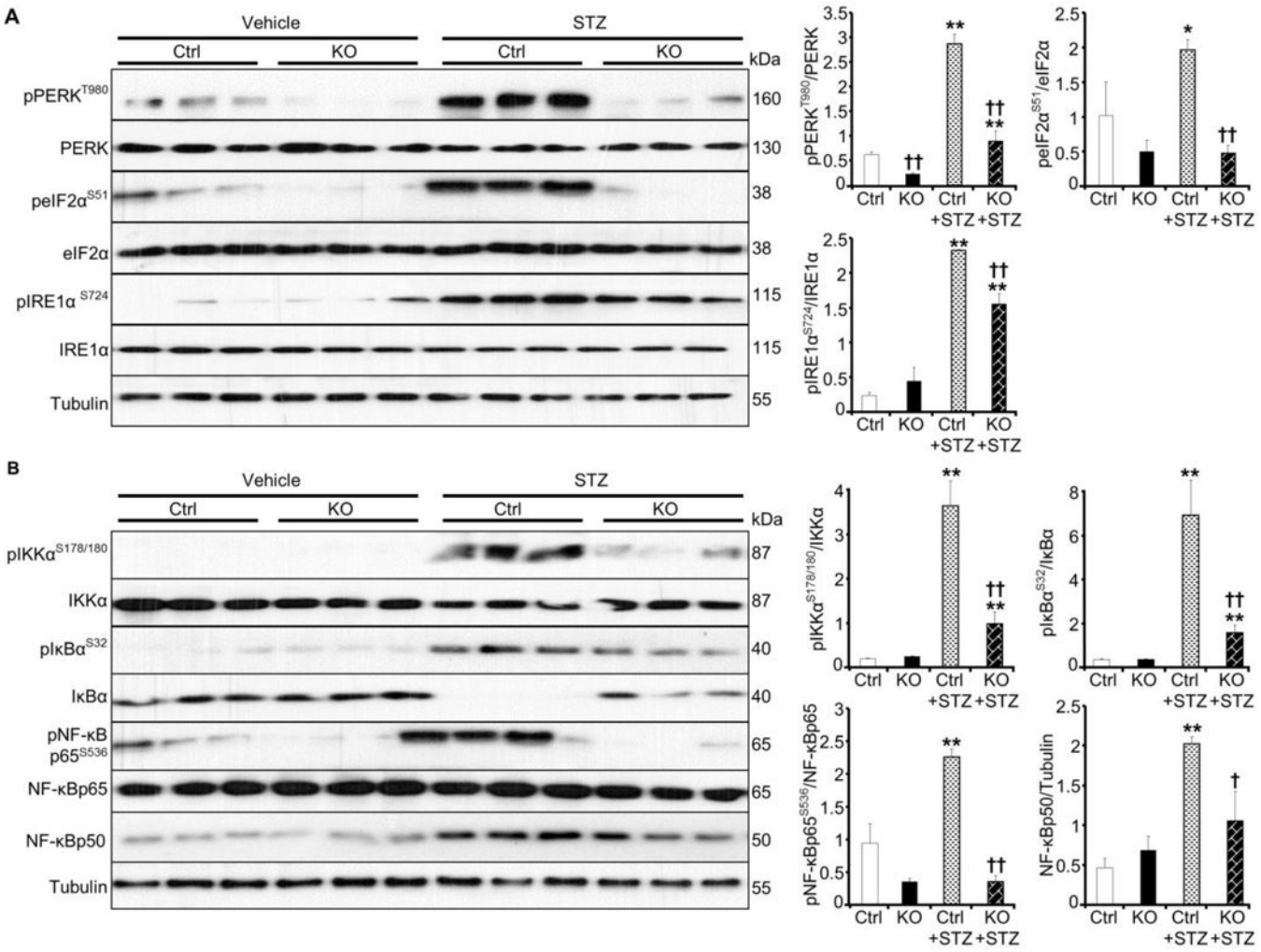

Figure 3. Decreased hyperglycemia-induced renal ER stress and inflammation in pod-sEHKO mice

Immunoblots of key proteins in (**A**) ER stress signaling: pPERK (Thr980), PERK, peIF2α (Ser51), eIF2α, pIRE1α (Ser724), IRE1α, and (**B**) NFκB signaling: pIKKα (S178/180), IKKα, pIκBα (S32), IκBα, pNF-κBp65 (S536), NF-κBp65, and NF-κBp50 in kidney lysates of control (Ctrl) and pod-sEHKO (KO) male mice without and with STZ at 24 weeks after injection (n=6/group/treatment). Each lane represents lysate from a different animal, and representative immunoblots are shown. Bar graphs of pPERK/PERK, peIF2α/eIF2α, pIRE1α/IRE1α, pIKKα/IKKα, pIKBα/IKBα, pNF-κBp65/NF-κB and NF-κBp50/Tubulin as means ± SEM. In **A** and **B**; *$p \leq 0.05$; **$p \leq 0.01$ indicate a significant difference between vehicle vs. STZ, and †$p \leq 0.05$; ††$p \leq 0.01$ indicate a significant difference between Ctrl vs. KO.

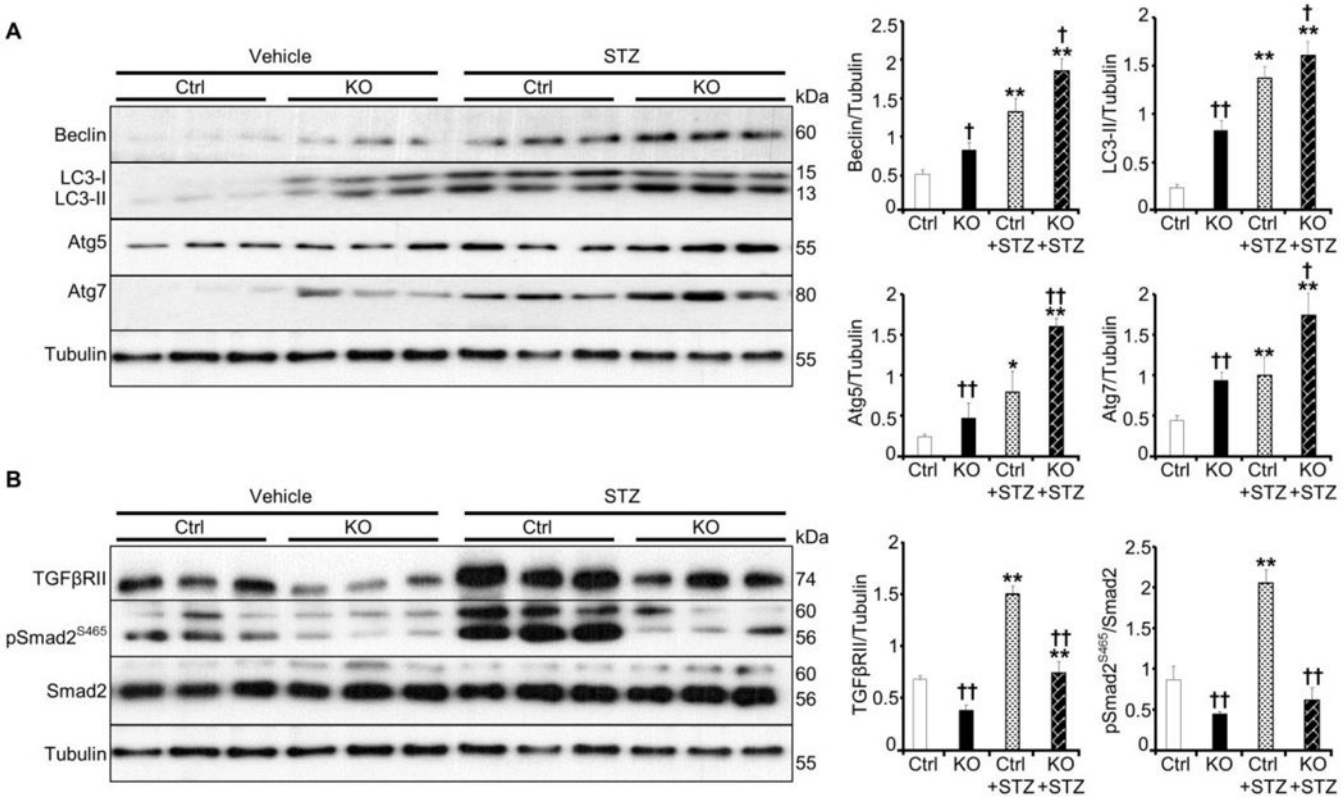

Figure 4. Modulation of renal autophagy and fibrosis in pod-sEHKO mice

Immunoblots of key proteins in (**A**) autophagy: Beclin, LC3-I/II, Atg5 and Atg7, and (**B**) fibrosis: TGFβRII, pSmad2 (S465) and Smad2 in kidney lysates of control (Ctrl) and pod-sEHKO (KO) male mice without and with STZ at 24 weeks after injection (n=6/group/treatment). Each lane represents lysate from a different animal, and representative immunoblots are shown. Bar graphs represent Beclin/Tubulin, LC3-II/Tubulin, Atg5/Tubulin, Atg7/Tubulin, TGFβRII/Tubulin and pSmad2/Smad2 as means ± SEM. In **A** and **B**; *$p \leq 0.05$; **$p \leq 0.01$ indicate a significant difference between vehicle vs. STZ, and †$p \leq 0.05$; ††$p \leq 0.01$ indicate a significant difference between Ctrl vs. KO.

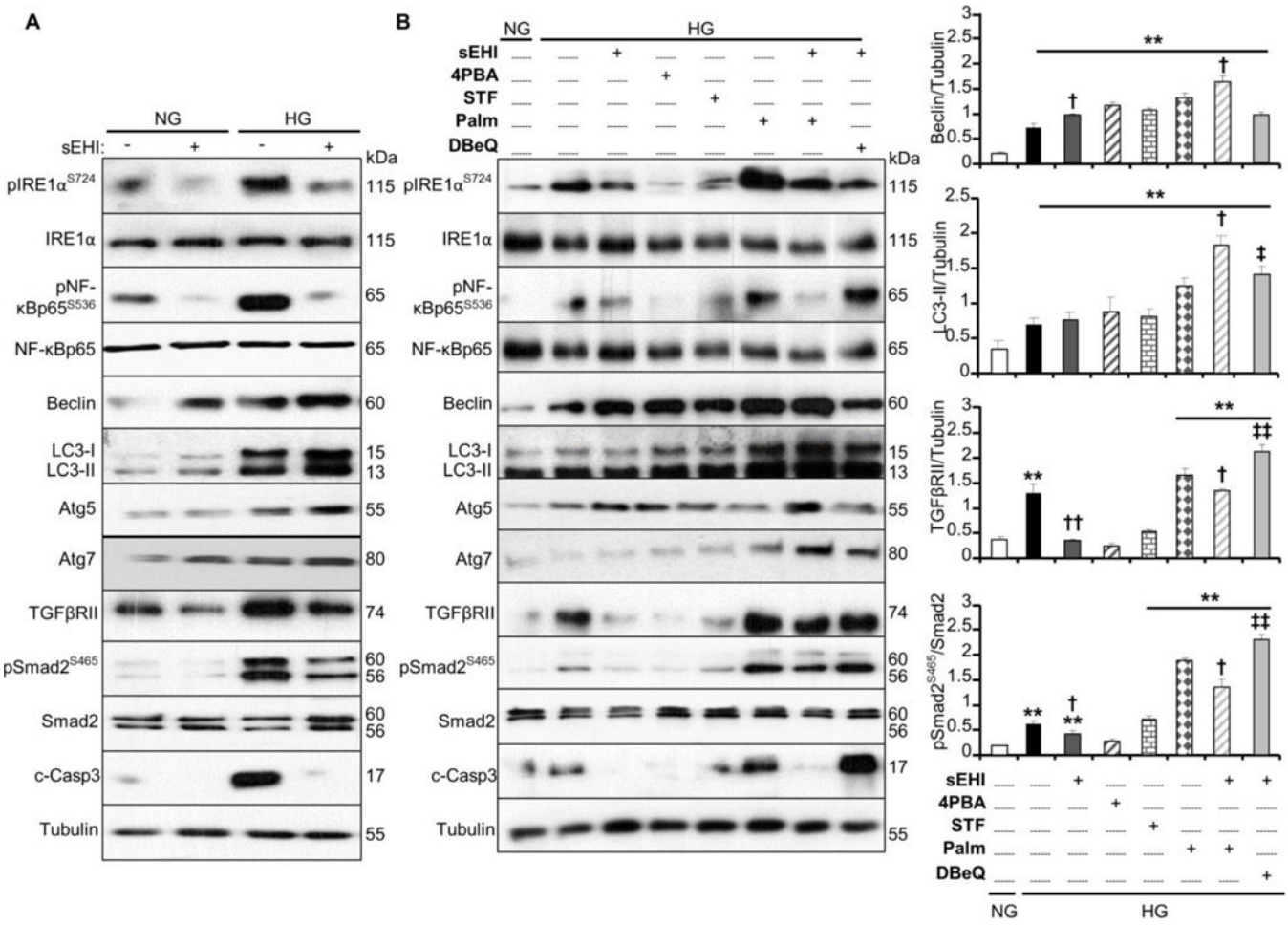

Figure 5. Pharmacological inhibition of sEH in immortalized podocytes attenuates ER stress and enhances autophagy under high glucose culture

A) Differentiated E11 podocytes were cultured in normal (NG; 5.6mM) and high glucose (HG; 25mM) for 72h without (−) and with (+) sEH pharmacological inhibition (sEHI; TPPU). Immunoblots of pIRE1α (Ser724), IRE1α, pNF-κBp65 (S536), NF-κBp65, Beclin, LC3-I/II, Atg5, Atg7, TGFβRII, pSmad2 (Ser465), Smad2, c-Caspase3, and Tubulin. Representative immunoblots from three independent experiments are shown. **B**) Immunoblots of differentiated E11 podocytes cultured in normal and high glucose treated with sEH inhibitor, chemical chaperone 4-phenylbutyrate (4PBA) to alleviate ER stress, autophagy inducer N-(3-Methylphenyl)-4-(4-pyridinyl)-2-thiazolamine (STF-62247), ER stress inducer Palmitate (Palm), and autophagy inhibitor N2,N4-dibenzylquinazoline-2,4-diamine (DBeQ). Representative immunoblots from three independent experiments are shown. Bar graphs represent Beclin/Tubulin, LC3-II/Tubulin, TGFβRII/Tubulin and pSmad2/Smad2 as means ± SEM. $^{**}p<0.01$ indicates a significant difference between NG vs. HG, and $\dagger p\leq 0.05$; $\dagger\dagger p\leq 0.01$ indicate a significant difference between sEHI-treated vs. non-treated cells cultured in HG media and exposed to the same treatment (+/−palmitate) (column 3 vs. column 2, and column 6 vs. column 5). $\ddagger p\leq 0.05$; $\ddagger\ddagger p\leq 0.01$ indicate a

significant difference between DBeQ-treated vs. non-treated cells cultured in HG media and exposed to sEHI (column 7 vs. column 3).