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Original Article

Diabetic conditions modulate the adenosine monophosphate-activated protein kinase of podocytes

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Abstract

Background: Adenosine monophosphate-activated protein kinases (AMPKs), as a sensor of cellular energy status, have been known to play an important role in the pathophysiology of diabetes and its complications. Because AMPKs are known to be expressed in podocytes, it is possible that podocyte AMPKs could be an important contributing factor in the development of diabetic proteinuria. We investigated the roles of AMPKs in the pathological changes in podocytes induced by high-glucose (HG) and advanced glycosylation end products (AGEs) in diabetic proteinuria.
Methods: We prepared streptozotocin-induced diabetic renal tissues and cultured rat and mouse podocytes under diabetic conditions with AMPK-modulating agents. The changes in AMPKα were analyzed with confocal imaging and Western blotting under the following conditions: (1) normal glucose (5mM, =control); (2) HG (30mM); (3) AGE-added; or (4) HG plus AGE-added.
Results: The density of glomerularphospho-AMPKα in experimental diabetic nephropathy decreased as a function of the diabetic duration. Diabetic conditions including HG and AGE changed the localization of phospho-AMPKα from peripheral cytoplasm to internal cytoplasm and peri- and intranuclear areas in podocytes. HG reduced the AMPKα (Thr172) phosphorylation of rat podocytes, and similarly, AGEs reduced the AMPKα (Thr172) phosphorylation of mouse podocytes. The distributional and quantitative changes in phospho-AMPKα caused by diabetic conditions were preventable using AMPK activators, metformin, and 5-aminoimidazole-4-carboxamide-1β-riboside.
Conclusion: We suggest that diabetic conditions induce the relocation and suppression of podocyte AMPKα, which would be a suggestive mechanism in diabetic podocyte injury.

Introduction

Diabetic nephropathy is one of the major complications of diabetes mellitus and currently the leading cause of end-stage renal disease worldwide [1]. Early clinical manifestations of diabetic nephropathy include hyperfiltration and microalbuminuria, which could progress to overt proteinuria and advanced renal injury. Accompanying morphological and

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ultrastructural changes include enlargement of glomeruli, mesangial expansion, thickening of the glomerular basement membrane, and effacement, denudation, and loss of podocytes [1]. Accompanying biochemical alterations with pathological changes lead to increases in the glomerular permeability as a result of the impaired glomerular filtration structure. These changes would be caused by hyperglycemia, glycated proteins, or irreversible advanced glycosylation end products (AGEs) via various mechanisms, including biochemical pathways, signaling, cytokines, and oxidative stress [2,3].

AMP (adenosine monophosphate)-activated protein kinase (AMPK) is a ubiquitously expressed heterotrimeric kinase highly conserved from yeast to plants and animals that plays a key role in the regulation of energy homeostasis by coordinating multiple metabolic pathways [4–6]. AMPK is composed of a catalytic α subunit and regulatory β and γ subunits, each of which is encoded by two or three distinct genes (α1, 2; β1, 2; γ1, 2, 3) [4–6]. The kinase is activated by an elevated AMP/adenosine triphosphate (ATP) ratio due to cellular and environmental stress, such as heat shock, hypoxia, and ischemia [4–6].During energy stress as the level of ATP begins to fall, there is a marked increase in the cellular concentrations of AMP [4–7]. This increase in AMP leads to the activation of AMPK via multiple mechanisms [7]. Once activated, AMPK acts to restore energy homeostasis by phosphorylating multiple substrates that act both to stimulate energy production and minimize energy consumption. The relationship between AMPK activation and the beneficial metabolic effects provides the rationale for the development of new therapeutic strategies for diabetic vascular diseases [8]. Thus, pharmacological AMPK activation may, through signaling and metabolic and genetic expression effects, reduce the risk of diabetic and metabolic complications.

Over the past decade, the biology and biochemistry of the AMPK pathway have been studied intensively in various organs, such as the liver, skeletal muscle, and heart. Although AMPK is abundantly expressed in the kidney [9,10], its role in renal physiology and disease is less understood compared with that in other organs [11,12]. In recent years, although interest in AMPK in the kidney has intensified, only a few studies have been conducted on the role of AMPKs in podocytes. We hypothesized that diabetic conditions would induce changes in AMPK in podocytes and that therapeutic modulation of AMPK would be a potential target in the treatment of diabetic podocytopathy.

Methods

Preparation of rat diabetic renal tissue

All animal experimental procedures were performed according to the guidelines for the care and use of animals established by Yeungnam University, Gyeongsan, Korea. Diabetes was induced by an intravenous injection of streptozotocin (STZ; Sigma Chemical Co., St. Louis, MO, USA) at 45 mg/kg, freshly dissolved in 0.1M sterile sodium citrate, pH 4.5, in 6-week-old rats weighing 180–220 g. The rats were considered diabetic if their blood glucose levels were above 200 mg/dL at 48 hours after STZ injection. Control rats were given an equivalent amount of saline via the tail vein. The rats were sacrificed 48 hours, 4 weeks, and 10 weeks after the induction of diabetes. Each group consisted of four animals. Both kidneys were removed and used for the immunofluorescence assessment.

Cell culture of rat glomerular epithelial cells and mouse podocytes

Rat glomerular epithelial cells (GEpCs), cloned from primary rat glomerular cultures by Kreisberg [13], were characterized by a sensitivity to puromycin amino nucleoside, positive staining for Heymann antigen (gp330) and podocalyxin, and negative staining for factor VIII [13–15]. GEpCs were maintained as previously described [15]. Experiments were performed with cells between passages 15 and 18. Conditionally immortalized mouse podocytes were kindly provided by Dr Peter Mundel (University of Harvard, Boston, MA, USA) and were cultured and differentiated for at least 2 weeks as described previously [16].

Preparation of culture additives and treatment conditions

Cells were serum-deprived to reduce the background 24 hours prior to each experiment and then exposed to glucose and/or AGEs. Rat GEpCs or mouse podocytes were incubated in culture medium containing either 5mM glucose (normal glucose) or 30mM glucose (HG) without insulin. AGEs were produced using the technique previously described by Ha et al [15]. To imitate the long-term diabetic condition, AGEs were added (5 μg/mL), and controls were established using unmodified bovine serum albumin (5 μg/mL). To exclude the effect of additionally produced glycated proteins during culturing, incubation did not last longer than 48 hours. The fetal bovine serum was reduced to 0.5% at the last medium change to reduce the background caused by any humoral effects of the serum prior to protein and RNA extraction. For identification purposes, AGEs and bovine serum albumin were denoted “A” and “B”, and glucose at 5mM and 30mM was denoted by “5” and “30”, respectively. The meaning of each condition has been described previously [15]. For AMPK activation, 5-aminoimidazole-4-carboxamide-1β-riboside (AICAR; Merck KGaA, Darmstadt, Germany) and metformin (Daewoong Pharmaceutical Co., Seoul, Korea) treatment was applied using concentrations of 0.5mM and 2mM, respectively, for 24 hours. For AMPK inhibition, compound C (Merck KGaA) was added to the medium at a concentration of 5μM for 24 hours.

Immunoblotting analysis for AMPK

The confluently grown cell layers were incubated with additives for various durations, extracted in protein extraction solution (PRO-PREP; Intron, Seongnam, Korea) containing phenylmethylsulfonyl fluoride, ethylendiamine tetraacetic acid (EDTA), pepstatin A, leupeptin, and aprotinin, and then the protein concentrations were determined as previously described [16]. For the immunoblotting assay, 25 μg of boiled extracts was applied to 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) gels and transferred to polyvinylidene fluoride membranes (Bio-Rad Laboratories, Hercules, CA, USA). Then, the membranes were air-dried and blocked in 3% fat-free milk prior to incubation with monoclonal rabbit antiphospho-AMPKα1/2 (Thr172) (Cell Signaling Technology, Danvers, MA, USA) and polyclonal rabbit anti-AMPKα1/2(Santa Cruz Biotechnology, Santa Cruz, CA, USA). As a loading control, anti-β-tubulin antibody (Santa Cruz Biotechnology) was also used. After incubation with horseradish peroxidase-conjugated secondary antibodies (Santa Cruz Biotechnology), bands were detected using the ECL