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Hyperuricemia induces hypertension through activation of renal epithelial sodium channel (ENaC)

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ARTICLE INFO

Article history:
Received 28 May 2015
Accepted 21 October 2015

Keywords:
Hyperuricemia
Epithelial sodium channel
ENaC regulatory complex
Hypertension

ABSTRACT

Objectives. The mechanisms leading to hypertension associated with hyperuricemia are still unclear. The activity of the distal nephron epithelial sodium channel (ENaC) is an important determinant of sodium balance and blood pressure. Our aim was to investigate whether the effect of hyperuricemia on blood pressure is related to ENaC activation.

Methods. A hyperuricemic model was induced in rats by 2% oxonic acid and 6 mg/dl uric acid (UA). The hyperuricemic rats were co-treated with either 10 mg/kg/d benzbromarone (Ben) or 1 mg/kg/d amiloride (Ami). Blood pressure was monitored using a tail-cuff, and blood, urine, and kidney samples were taken. Western blotting and immunohistochemical staining were performed to determine the expressions of ENaC subunits and components of the ENaC Regulatory Complex (ERC) in kidney tissue or mCCD cells.

Results. Serum uric acid (SUA) was increased 2.5–3.5 times above normal in hyperuricemic rats after 3 weeks and remained at these high levels until 6 weeks. The *in vivo* rise in SUA was followed by elevated blood pressure, renal tubulointerstitial injury, and increased expressions of ENaC subunits, SGK1, and GILZ1, which were prevented by Ben treatment. The decrease in urinary Na^+ excretion in hyperuricemic rats was blunted by Ami. UA induced the expression of all three ENaC subunits, SGK1, and GILZ1, and increased Na^+ transport in mCCD cells. Phosphorylation of ERK was significantly decreased in both UA-treated mCCD cells and hyperuricemic rat kidney; this effect was prevented by Ben co-treatment.

Conclusion. Our findings suggest that elevated serum uric acid could induce hypertension by activation of ENaC and regulation of ERC expression.

Abbreviations: ENaC, epithelial sodium channel; CCD, cortical collecting duct; SGK1, serum- and glucocorticoid-induced kinase; GILZ, glucocorticoid-induced leucine zipper protein; E3, ubiquitin-protein isopeptide ligase; ERC, ENaC Regulatory Complex; mCCD, murine CCD; UA, uric acid; ALD, aldosterone; BUN, blood urea nitrogen; Cr, creatinine; FENa, filtration sodium excretion fraction; FEK, filtration potassium excretion fraction; CCr, creatinine clearance rate.

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http://dx.doi.org/10.1016/j.metabol.2015.10.026
0026-0495/

1. Introduction

In the past decades, accumulating evidence obtained from experimental animal studies, clinical trials, and epidemiological investigations has indicated an association between hyperuricemia and the development of hypertension and renal injury [1]. Epidemiological studies show that an estimated 25–50% of hypertensive individuals are hyperuricemic [2]. The development of hyperuricemia-induced hypertension can be divided into two phases [3–5]. In the first phase, uric acid activates the renin–angiotensin system and diminishes nitric oxide production, leading to endothelial dysfunction and vasoconstriction. In the second phase, as microvascular disease and tubulointerstitial inflammation develop in the kidney, the hyperuricemic hypertension becomes salt sensitive [6–8]. However, the causal mechanisms of hyperuricemia effects on hypertension are still poorly understood.

The amiloride-sensitive epithelial sodium channel (ENaC) controls the rate-limiting step for sodium reabsorption in epithelial cells, which cover the distal part of the renal tubule, the distal colon, the duct of several exocrine glands, and the lung. ENaC localizes to the apical membranes of principal cells in the distal nephrons, where it is responsible for the fine control the Na^+ reabsorption in those nephrons. ENaC dysregulation has been implicated in many other clinical conditions, including pulmonary edema, acute respiratory distress syndrome, and nephrosis, as well as salt-dependent hypertension [9,10]. Structurally, ENaC is comprised of α, β, and γ homologous subunits, and is highly regulated by various hormonal pathways, such as aldosterone, vasopressin, and insulin in the kidney [11]. Serum- and glucocorticoid-induced kinase (SGK1) and glucocorticoid-induced leucine zipper protein (GILZ) are the predominant molecules that mediate the hormonal regulation of ENaC through the inhibition of ENaC degradation [12,13]. Both SGK1 and GILZ can inhibit ENaC phosphorylation and its subsequent degradation by deactivating the ERK pathway and interfering with the action of the ENaC E3 ligase Nedd4-2 [14].

The findings that about 70% of uric acid is excreted by kidneys and that hyperuricemia is implicated as a cause of salt-sensitive hypertension and kidney disease led to our demonstration [15], in the current study, that the treatment with uric acid (UA) caused elevated blood pressure accompanied by renal tubulointerstitial injury and increases in expressions of ENaC subunits, SGK1, and GILZ1 in the rat kidney. Meanwhile, the phosphorylation of ERK was significantly decreased in both UA-treated mCCD cells and the hyperuricemic rat kidney. The changes in blood pressure, the expressions of ENaC subunits, and components of ERCs were prevented by the uricosuric agent benzbromarone (Ben).

2. Material and Methods

2.1. Reagents and Antibodies

All reagents were obtained from Sigma-Aldrich (St. Louis, MO) unless otherwise stated. A rabbit polyclonal antibody targeting an epitope at the extracellular loop of α-ENaC has been described [16]. Monoclonal anti-β-ENaC and antibodies specific for GILZ1, P-ERK, and ERK were purchased from Santa Cruz Biotechnology, Santa Cruz, CA; anti-γ-ENaC antibody was obtained from Stress-Marq, Victoria, BC. Polyclonal anti-SGK1 was obtained from Upstate, Temecula, CA. Other antibodies or reagents used included secondary antibodies against mouse or rabbit (GE Healthcare, Piscataway, NJ), U0126, ERK1/2 inhibitor (Calbiochem, San Diego, CA).

2.2. Animal Models

Adult male Sprague–Dawley rats weighing 180–200 g were acquired from the Laboratory Animal Center of Nanjing Medical University. The rats were housed under a 12 h light/dark cycle at a temperature of 24 °C and relative humidity of 56 ± 10%, with free access to water and normal diet (22% protein). Rats were randomly assigned to six groups (n = 5 per each group) after measuring body weight, blood pressure (BP), blood urea nitrogen (BUN), creatinine (Cr), and uric acid: 1) Control group (Con) fed with normal rat chow; 2) Oxonic acid (OA) group fed with a diet containing 2% oxonic acid (OA) and drinking water containing 6 mg/dl uric acid; 3) OA + benzbromarone group (OA + Ben) that received the OA treatment combination together with 10 mg/kg/d benzbromarone dissolved in the drinking water; 4) OA + amiloride group (OA + Ami) that received the OA treatment combination together with 1 mg/kg/d amiloride dissolved in the drinking water; 5) Benzbromarone group (Ben) fed the normal diet, together with 10 mg/kg/d benzbromarone dissolved in the drinking water; 6) Amiloride group (Ami) fed the normal diet, together with 1 mg/kg/d amiloride dissolved in the drinking water. Serum uric acid (SUA), aldosterone (ALD), blood urea nitrogen (BUN), creatinine (Cr), electrolyte levels including sodium (Na^+), potassium (K^+), and chloride (Cl^-), and urine creatinine were measured at weeks 0, 3, and 6 in the core lab of the Second Affiliated Hospital of Nanjing Medical University. Urinary volume, food and water intake, urinary Na^+, and urinary K^+ were measured. The filtration sodium excretion fraction (FENa), filtration potassium excretion fraction (FEK), creatinine clearance rate (CCr), and urinary Na^+/K^+ ratio were calculated at weeks 0, 3, and 6. Systolic blood pressure (SBP) was assessed at weeks 0, 2, 4, and 6. At the end of 6 weeks, all rats were sacrificed; blood and kidney samples were obtained at time of sacrifice. One part of the kidneys was fixed in 10% phosphate-buffered formalin, followed by paraffin embedding, for histological studies. The remaining kidney tissues were snap-frozen in liquid nitrogen and stored at −80 °C for protein extraction.

All animal experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Usage Committee of Nanjing Medical University (Approval code: NJMU-IACUC 20130101).

2.3. Analysis of Kidney Functions

Systolic blood pressures were measured using a standard tail-cuff method (BP-2000, Visitech Systems) in unanesthetized, prewarmed rats at an ambient temperature of 29 °C. All animals were preconditioned for BP measurements 1 week before experiment. Their SUA, ALD, BUN, Cr, and electrolyte