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Short communication

Reduced renal ClC-5 Cl^- channel expression in spontaneously hypertensive rats with microalbuminuria

Karo Tanaka*, Toshio Nakaki

Department of Pharmacology, Teikyo University School of Medicine, 2-11-1 Kaga, Itabashi, Tokyo, 173-8605, Japan

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Abstract

Mutations in a renal-specific Cl^- channel, ClC-5, result in low-molecular-weight proteinuria. Herein we studied ClC-5 expression in the kidneys of spontaneously hypertensive rats (SHR) to identify possible causes of their increased urinary excretion of albumin. The amount of ClC-5 protein was significantly reduced in 3-month-old SHR as compared with normotensive Wistar/Kyoto (WKY) rats. The ClC-5 protein level was partially restored by short term administration of perindopril, an inhibitor of angiotensin-converting enzyme. Corresponding to the increase in ClC-5 expression, the albuminuria in SHR improved to the control level. These results implicate the ClC-5 Cl^- channel reduction in the development of albuminuria in the early stage of essential hypertension.

Keywords: ClC-5 Cl^- channel; Proteinuria; Hypertension; Angiotensin-converting enzyme; Perindopril

1. Introduction

Microalbuminuria, an abnormal elevation of urinary albumin excretion less severe than clinical albuminuria, is now an accepted predictive marker for early detection of cardiovascular complications of hypertension with or without diabetes (Gerstein et al., 2001; Leoncini et al., 2002). It is also highly predictive of the development of diabetic nephropathy, but the prognostic value of microalbuminuria for the risk of hypertensive nephropathy has not been confirmed by prospective studies (Crippa, 2002). In order to establish microalbuminuria as a reliable marker for the risk of renal insufficiency in essential hypertension, it is necessary to identify its causative factors and to fully understand its pathogenesis.

Microalbuminuria presents in about 25% of patients with essential hypertension. It is associated with higher renal vascular resistance, higher renin system activities, higher creatinine levels and greater drops in glomerular filtration rate over time as compared with normoalbuminuric hypertension (Redon, 1998). Several clinical experiments demonstrated proteinuria to be reduced concomitantly with blood pressure when antihypertensives were administered to diabetic and non-diabetic patients with renal disease (Maki et al., 1995). The antiproteinuric effect of angiotensin-converting enzyme inhibition appeared, however, to be greater than that of other agents despite their comparable capacity to reduce blood pressure (Gansevoort et al., 1995; Maki et al., 1995).

Increased transglomerular passage of albumin, resulting from increased intraglomerular pressure and permselectivity changes of the glomerular filter, has been suggested to be the major mechanism of albuminuria in essential hypertension (Ljungman, 1990). In contrast, no specific pathological kidney change has been identified in most patients with primary hypertension and microalbuminuria. One possible cause of milder proteinuria, characterized by the excretion of albumin and low-molecular-weight proteins, is defective reabsorption by proximal tubular cells through receptor-mediated endocytosis. There is no evidence to date showing involvement of the tubular component in the microalbuminuria associated with essential hypertension.

* Corresponding author. Tel.: +81 3 3964 1211; fax: +81 3 3964 0602.
E-mail address: karo@med.teikyo-u.ac.jp (K. Tanaka).

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A renal-specific Cl^- channel, ClC-5, is predominantly expressed on endocytotic vesicles of the renal proximal tubule, co-localizing with H^+-ATPase to provide the electrical counterbalance for proton transport (Günther et al., 1998). Since acidification of these vesicles is necessary for the endocytotic process, functional loss of the ClC-5 channel results in renal excretion of low-molecular-weight proteins and albumin in patients with Dent's disease (Lloyd et al., 1996). In order to identify a tubular factor related to the presence of hypertensive microalbuminuria, we investigated the expression of the ClC-5 Cl^- channel in spontaneously hypertensive rats (SHR), as well as the protective effect of angiotensin-converting enzyme inhibition against microalbuminuria.

2. Materials and methods

2.1. Animals and drugs

Eight week-old male SHRs (SHR/Izm, Funabashi Farm, Chiba, Japan) were assigned to receive vehicle or perindopril at 2.0 mg/kg/day (n=6). Perindopril dissolved in water was orally administered once a day for up to 6 weeks. Age- and sex-matched Wistar–Kyoto (WKY) rats (WKY/Izm, Funabashi Farm) were used as a normotensive control. All rats were maintained at a constant temperature (23–25 °C) with a 12–12 h light/dark cycle. Standard rat chow and tap water were supplied freely. Blood pressure was monitored using a tail-cuff probe connected to a pressure monitor (BP-98A, Softron). Procedures involving animals and their care were conducted according to the Guiding Principles for the Care and Use of Laboratory Animals approved by the Institutional Ethics Committee on Animal Experimentation.

2.2. Determination of urinary proteins

Twenty-four-hour urine samples were collected by using metabolic cages for four sequential days in the third week of perindopril treatment (n=6). Total protein concentrations in urine samples were measured using the Protein Assay System (Bio-Rad). The individual protein components between 15,000 and 120,000 Da in the urine samples were separated by molecular weight with sodium dodecyl sulfate polyacrylamide gel and visualized with silver staining (Daiichi Kagaku). Albumin solutions of known concentrations were stained as a standard, and the digital density was converted to the albumin concentration using the computer program NIH image (version 2.6.1) to determine the albumin excretion rate.

2.3. Expression analysis of ClC-5 in the renal cortex

Perindopril treated/untreated SHRs were sacrificed at the end of the third and sixth weeks of drug administration (11 and 14 weeks of age, n=3) together with the WKY controls. Renal cortex tissues were divided in half and subjected to protein and RNA extraction. For Western blot analysis, crude membrane fractions were prepared according to a method described elsewhere (Günther et al., 1998), electrophoresed using a Phast Gel System (Pharmacia) and subjected to the standard procedure for immunoblotting using ECL-plus Western blotting detection system (Amersham Lifescience). The dilution factor for anti-rat ClC-5 antibody was 1:2500. For Northern blot analysis, total RNA was extracted using a Qiagen RNeasy Protect Kit (Qiagen) and a radiolabeled ClC-5 cDNA fragment was probed to detect mRNA expression.

2.4. Statistics

Values are expressed as mean±S.E.M. Two sample Student's t tests or Mann–Whitney's U test were used to evaluate statistical significance, as appropriate. P values less than 0.05 were considered statistically significant.

3. Results

Between 8 and 14 weeks of age, when perindopril was administered, systolic blood pressure was significantly higher in the SHR group than in the WKY group. Treatment with perindopril at an antihypertensive dose (2 mg/kg/day) (Chiba et al., 1994) reduced blood pressure in the SHR group to a level similar to that in the WKY group (Fig. 1A). Angiotensin-converting enzyme inhibition with perindopril for 1 week effectively reduced blood pressure in SHR to the control level.

Twenty-four-hour urine samples were collected and total protein amount was quantitated with a modification of Bradford's method. There was a slight increase in the total protein excretion rate of the SHR groups, but the differences did not reach statistical significance (Fig. 1A). In contrast, detailed analysis of protein components by electrophoresis revealed albumin excretion increases exceeding 10-fold in the SHR group urine as compared with that of the WKY control group (Fig. 1B). Three weeks of angiotensin-converting enzyme inhibition with perindopril reduced the albuminuria in SHRs to the control level. The protective effect of perindopril against albuminuria was observed during the first week of perindopril treatment.

There was no overt difference in gross appearance of kidney sections among these three groups. Morphologic injury, which was assessed by the percentages of glomerular crescents and hypercellularity, was absent on light microscopy. Serum concentrations of urea nitrogen and creatinine in the three groups were identical and all within normal range (data not shown).

Western blot analysis of ClC-5 protein in the renal cortex revealed ClC-5 expression to be lower in the SHR group than in WKY rats at 11 and 14 weeks of age. The relative expressions of ClC-5 protein in SHR were 40.2±7.6%

A

	WKY	SHR	SHR + perindopril
Systolic blood pressure (mmHg)	132.0 ± 6.4	155.3 ± 1.9	134.8 ± 3.9*
Total urinary protein (mg/kg/24h)	304.2 ± 9.7	318.3 ± 28.1	372.4 ± 20.8

B

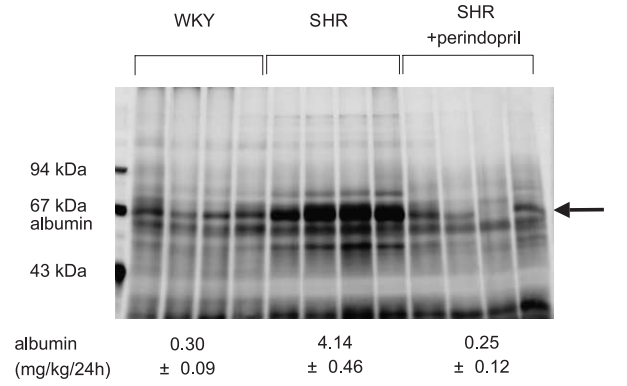

Fig. 1. Reduction of high blood pressure and microalbuminuria in SHR by angiotensin converting enzyme inhibition for 3 weeks. (A) Total urinary protein was only slightly increased by perindopril treatment (n=6). *P<0.01 vs. vehicle-treated SHRs. Data are expressed as means±S.E.M. (B) Urinary low-molecular-weight protein profile from the SHR groups and the WKY group. All lanes were loaded with a urine sample at a volume normalized by creatinine concentrations, and proteins were visualized by silver staining. Each lane represents one sample, each obtained from a different rat. The elevated excretion of urinary proteins, consisting mainly of albumin in the SHR group (indicated by arrow), improved to the control level with perindopril treatment.

(Fig. 2A) and 28.1±2.1% (data not shown) of those in the control WKY at 11 and 14 weeks of age, respectively. This reduction was partially reversed concomitantly with the correction of blood pressure by perindopril treatment for 3 (Fig. 2A) or 6 weeks (data not shown). In contrast, the ClC-5 mRNA level in SHRs was double that in WKY rats but perindopril treatment reduced ClC-5 mRNA expression to the WKY level (Fig. 2B).

4. Discussion

In this study, we investigated a possible cause of microalbuminuria as an early sign of hypertensive nephropathy progression and showed that the renal-specific chloride channel ClC-5 is significantly reduced in the renal cortex of microalbuminuric SHRs. The reduction appeared to be progressive. Thus, the relative expression of ClC-5 protein in SHRs was 40% of that in controls at 11 weeks of age and was less than 30% of that at 14 weeks. To exclude the glomerular lesion having resulted from hypertensive organ damage causing albuminuria, we employed SHR not yet at hypertensive age when there are no renal lesions (Feld et al., 1977). Eight to 14 week-old SHRs manifested isolated albuminuria, which was absent in normotensive WKY controls. Detailed urinalysis revealed no proteins equal in size to or larger than γ-globulin, the existence of which would indicate proteinuria resulting from loss of the glomerular barrier for protein filtration. Renal function in these rats was proven to be normal with respect to serum urea, creatinine and total protein levels. Therefore, overall renal function in SHR at the stage we examined was normal and no glomerular lesion was evident. This suggests that the observed microalbuminuria in young SHRs might be related to ClC-5 Cl^- channel loss, which would account for reduced endocytosis of albumin in proximal tubules. In humans, female carriers of Dent's disease, who are 50% chimeric for the ClC-5 mutation in somatic tissues, manifest low-molecular-weight proteinuria (Wrong et al., 1994). Hence, the 60–70% ClC-5 protein reduction in SHR can reasonably be suggested to underlie the observed microalbuminuria.

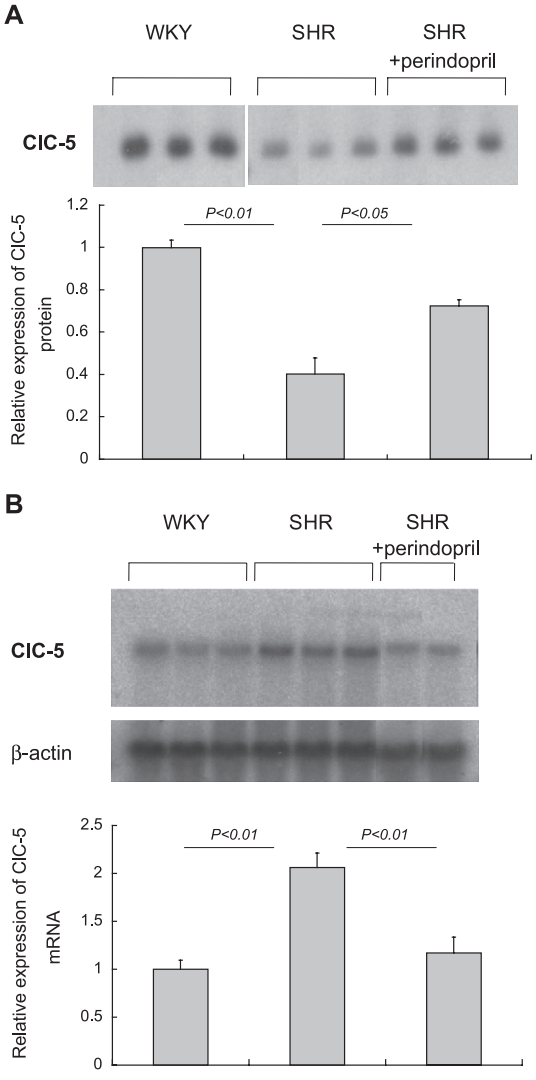

Fig. 2. (A) Immunoblot analysis of the renal ClC-5 Cl^- channel. Crude membrane fraction were isolated from kidneys of SHR treated or untreated with perindopril for 3 weeks (n=3). The data represent the ClC-5 protein expression in the SHR renal cortex in comparison to the control WKY. (B) Northern analysis of the renal ClC-5 Cl^- channel. The data represent ClC-5 mRNA expression in the same tissues as described above. All data are expressed as means±S.E.M.

Blood pressure lowering by angiotensin-converting enzyme inhibition was associated with partial recovery of the ClC-5 protein level, while microalbuminuria disappeared completely. Therefore, the observed microalbuminuria in SHR may have two independent additive causes: hyperfiltration of albumin due to increased glomerular capillary pressure and reduced tubular reabsorption due to dysfunction of the endocytotic pathway.

ClC-5 mRNA was, on the other hand, increased in the SHR renal cortex, possibly as a response to the increased urinary albumin load. Therefore, the decrease in ClC-5 protein is attributable to reduced translational activity or impaired stability of the polypeptide in the renal cortex. Scherer et al. (2001) previously reported only ClC-5 mRNA levels in various strains of rats with hypertension and other cardiovascular diseases. Our finding of decreased ClC-5 protein with increased ClC-5 mRNA expression raises the necessity of also monitoring expression patterns at the protein level to study ClC-5 involvement in different pathophysiological conditions.

The renal protective effects of angiotensin-converting enzyme include prevention of glomerular injury, via dilation of renal efferent arterioles, increased permselectivity of the filtering membrane, and reduction of mesangial cell proliferation and matrix production by minimizing exposure of the mesangium to proteinaceous factors. Alleviation of the oxidative stress which accompanies hypertension may also be important (Agarwal et al., 2004). An increased state of oxidative stress, indicated as an increased lipid peroxidation, was demonstrated in SHR proximal tubules (White and Sidhu, 1998), which is presumably responsible for a functional nitric oxide deficiency (Welch et al., 2000). Since chronic inhibition of nitric oxide synthase in vivo results in hypertension and proteinuria (Zatz and Baylis, 1998), an imbalance in the productions of reactive oxygen species and nitric oxide may underlie the pathophysiology of essential hypertension. Different antioxidants are shown to be beneficial for hypertensive conditions, as exemplified by phenidone protecting against proteinuria in stroke-prone spontaneously hypertensive rats (Munsiff et al., 1992), and low plasma concentrations of both endogenous and diet-derived antioxidants are shown to be associated with microalbuminuria in humans (Giner et al., 2004; Rowley et al., 2003). While administration of angiotensin II enhances oxygen radical generation in rat renal proximal tubules and increases systolic blood pressure and proteinuria (Haugen et al., 2000), angiotensin-converting enzyme inhibition and angiotensin II receptor blockade could attenuate the oxidative stress in vivo (Welch and Wilcox, 2001; de Cavanagh et al., 2003). Although there is no direct evidence showing that increased oxidative stress in proximal tubules results in ClC-5 channel reduction, an outward chloride current was reportedly modulated by oxidants and antioxidants in human retinal pigment epithelium cells which express ClC-2, -3, -5 and CFTR mRNA (Weng et al., 2002). We speculate that the restoration of ClC-5 protein in the kidneys of SHR by angiotensin-converting enzyme inhibition could result from an improvement in oxidative stress.

Recent investigations have provided evidence that the proteinuria is not only a result of nephropathy but also a cause of further tubular and interstitial lesion development, exacerbating the existing nephropathy (Remuzzi and Bertani, 1998). From this perspective, some of the renoprotective effects of angiotensin-converting enzyme inhibition might be accomplished by reducing proteinuria via the amelioration of ClC-5 protein expression, leading to prevention of further renal injury. Taken together, our results demonstrate that reduced tubular reabsorption results from the reduction of ClC-5 protein in the hypertensive condition, possibly accounting for the development of microalbuminuria in the early stage of hypertension of SHR.

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