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Response of Renal Intercalated Cells to Dietary Potassium Intake

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Abstract

Compared to ancient humans who consumed enough potassium (K^+), modern humans seem to have taken in inadequate K^+. The renal collecting duct plays an important role in regulating K^+ homeostasis. There are at least three different types of cells in the collecting duct. The purpose of this study is to examine the effects of dietary K^+ intake on collecting duct intercalated cells. C57BL/6 mice had free access to a control (1%), low (<0.01%), or high (5%) K^+ diet for 1 week. Kidney tissue were processed for immunohistochemistry, light microscopy, and image analysis. Excessive dietary K^+ intake or deficiency significantly changed blood K^+ concentrations in mice. The size of type A intercalated cells increased by about 1.6 times in the low K^+ group and decreased in the high K^+ group. Conversely, the size of type B intercalated cells decreased in the low K^+ group and increased in the high K^+ group. The response of NANB cells to dietary K^+ intake was generally similar to that of type B cells. These results demonstrated that renal intercalated cells vary greatly with dietary K^+ intake. The structural changes in response to K^+ imbalance may be closely related to cardiovascular and renal disorders in modern humans.

Keywords

Kidney
Collecting duct
Potassium
Modern human

1. Introduction

Potassium (K^+) is an essential electrolyte that must be consumed for the normal functioning of nerves and muscles. In mammals, the concentration of potassium in extracellular fluids, including blood, remains relatively constant at 3.8–5 mmol/L and intracellularly at 120–140 mmol/L [1]. The intestines and kidneys play an important role in the absorption and excretion of ingested potassium [1].

While prehistoric humans consumed large amounts of potassium, ranging from about 150–290 mmol/day, modern humans on average consume only about 70 mmol/day [2-3]. The reason modern humans have a significantly lower potassium intake than prehistoric

humans is due to changes in dietary habits. Agricultural cultures began around 10,000 years ago, ushering in the era of low intake of potassium-rich fruits and plants. In general, the potassium content of food is greatly depleted during cooking and processing. Instead, sodium (Na^+) intake has increased excessively. Due to the salty nature of Korean food, the sodium intake of Koreans exceeds the recommendations of World Health Organization (WHO), while their potassium intake remains low [4]. Sodium and potassium are chemically similar monovalent cations, and their relative proportions are strongly associated with cardiovascular disease [5-6]. High sodium concentrations or potassium deficiency can lead to hypertension and are also associated with chronic kidney disease and urolithiasis [2,6-11]. Conversely, potassium supplementation in hypertensive patients can lower blood pressure [9,12].

The kidneys play an important role in regulating potassium homeostasis [1,13]. Briefly, potassium filtered through the glomerulus of the kidney is mostly reabsorbed in the proximal tubule, while urinary excretion of potassium is regulated by active secretion from the collecting duct [13]. The mechanism of potassium secretion from the collecting duct involves the co-operation of sodium/potassium-ATPase (Na+/K+-ATPase) and the epithelial sodium channel (ENaC) in the principal cell [14]. In a rather complex process, the lumen of the collecting duct develops a negative voltage, creating favorable conditions for the secretion of the potassium ion. In addition to the main cells, there are also intercalated cells that regulate acid-base secretion. Given the same favorable conditions for potassium secretion, intercalated cells can also secrete another cation, that is, acid (H^+).

Several previous studies have suggested that acid-base balance may be involved in potassium regulation in the kidney [15-16]. Interstitial cells of collecting duct can be broadly categorized into two types: acid-secreting type A cells and base-secreting type B cells, with the existence of a new subtype, the non A-nonB (NANB) cell, which has been recently recognized [17]. To date, the distinction between these subtypes has been based on immunohistochemistry using antibodies to transporters involved in acid-base secretion and microstructural differences detected through electron microscopy [17-19]. While type A cells are present in the outer surface, outer parenchyma and upper part of the inner parenchyma of the kidney, type B and NANB cells are distributed only in the outer surface. The role of NANB-type cells is not clearly understood to date.

The aim of the present study was to investigate the effect of potassium intake, which is strongly associated with health in anthropological studies, on the morphology of intercellular mesangial cells in different kidneys.

2. Materials and methods

2.1. Experimental animals

Thirty male C57BL/6 mice weighing 25–30 g were used. The experimental groups were fed a low-potassium diet ($K^+ < 0.01\%$, TD. 120441, Harlan Laboratory, Madison, WI, USA) or a high-potassium diet (K^+ 5%, TD. 110866, Harlan Laboratory, Madison, WI, USA) for 1 week. The control group received a normal diet (K^+ 1%, Harlan Laboratory, Madison, WI, USA) for the same period. Water was not restricted during the experimental period. Potassium concentrations were measured from the blood withdrawn from dorsalis pedis artery before animal sacrifice.

All animal experiments were approved by the Institutional Animal Care and Use Committee of Ewha Womans University (EWHA MEDIACUC 21-017-t).

2.2. Tissue sectioning

Animals were anaesthetized with 1.5% isoflurane (Abbott Laboratories, Queenborough, Kent, UK) and perfused with paraformaldehyde-lysine-periodate (PLP) via the abdominal aorta for 10 minutes. Kidneys were sectioned at the center into 1–2 mm thick sections and fixed for a further 16 hours at 4°C. The fixed tissue was

Table 1. Serum sodium and potassium concentration

	Control	Low K^+	High K^+
Na^+ (mmol/L)	144.1 ± 2.08	141.2 ± 2.95	142.8 ± 2.86
K^+ (mmol/L)	4.3 ± 0.41	2.6 ± 0.53*	5.0 ± 0.38*

Values represent the mean ± SD. *$P < 0.05$.

subjected to alcohol dehydration and xylene solution and embedded in paraffin.

2.3. Antibodies

As used in previous studies, anion exchanger 1 (AE1, Alpha Diagnostics, San Antonio, TX, USA) and H^+-ATPase (sc-19554, Santa Cruz Biotechnology, CA, USA) were used as markers of type A pseudocytes [19,20]. Pendrin (kindly provided by Dr. Soren Nielsen, Aarhus University, Denmark and in-house) was used as a marker for type B and NANB interphase cells [19].

2.4. Immunohistochemistry

Kidney tissues were cut into 4 μm thick sections and treated with 1.4% methanolic H_2O_2 for 30 minutes to remove endogenous peroxidase. After treatment with normal serum for 30 minutes, the primary antibodies were diluted 1:200 to 1000 and reacted overnight at 4°C. The next day, secondary antibodies (peroxidase conjugated donkey anti-rabbit IgG, Fab fragments, Jackson ImmunoResearchLaboratory, West Grove, PA, USA) diluted 1:200 were reacted for 1 hour. The color development was performed with a mixture of diaminobenzidine (DAB, Dako Omnis, Denmark). For double staining, the Vector SG kit (Vector Laboratories, Burlingame, CA, USA) was used, which has a blue color, contrasting with the brown of DAB. After mounting and alcohol dehydration, the sections were observed under a light microscope. Cell size was measured with an image analysis program (ImageJ, NIH, Bethesda, MD, USA), and statistical analysis was performed using GraphPad Prism (La Jolla, CA, USA) as previously reported [21]. Kidney tissues from 10 animals from the control, low-potassium, and high-potassium diets were used for cytometry for image analysis. Five collecting ducts were located per tissue section obtained from each animal, and the number of podocytes was counted by selecting those with at least five cells per collecting duct. Statistical analysis was performed using the *t*-test (parametric, unpaired, two-tailed), and the results were regarded as significant if the probability of significance (*P*-value) was less than 0.05.

3. Result

3.1. Weight and blood tests

The body weight of the animals in the low-potassium diet group decreased by approximately 10%, and no significant change was observed in the high-potassium diet group. Blood potassium concentration was significantly decreased in the low-potassium diet group (2.6 ± 0.53 mmol/L) compared to the control group (4.3 ± 0.41 mmol/L) and increased in the high-potassium diet group (5.0 ± 0.38 mmol/L) (Table 1). Blood sodium concentration was not significantly different among the experimental groups.

3.2. Histological changes

In the low-potassium diet group, the collecting duct epithelial cells were markedly hypertrophied, and the number of cells tended to increase, as previously reported [20-22]. Some cells were also observed to have two nuclei (Figure 1b). In the high-potassium diet group, the collecting duct cells appeared atrophied and the cell nuclei tended to stain rather darkly with hematoxylin (Figure 1c).

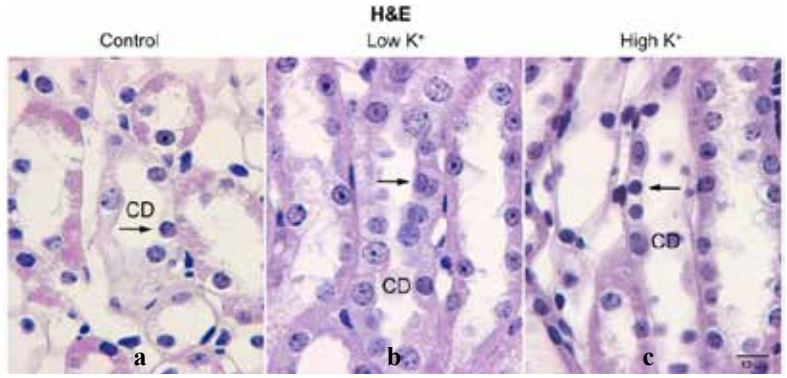

Figure 1. H&E staining. Collecting duct epithelial cells (arrows) were significantly enlarged in the low K^+ diet group when compared to control (a,b). Note that some cells have two nuclei (b). In the high K^+ diet group, the nuclei tended to stain somewhat darkly with hematoxylin in some cells (c). CD: collecting duct.

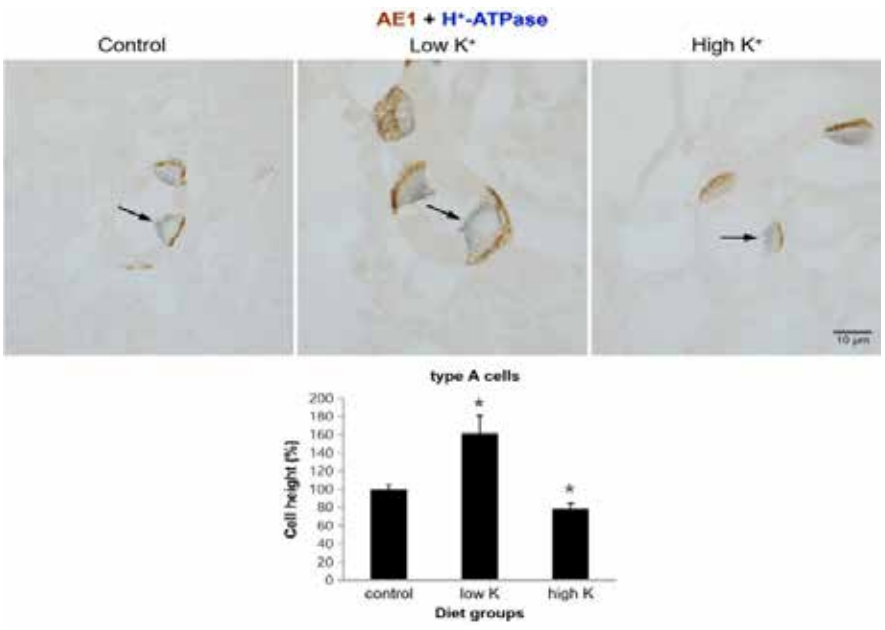

Figure 2. Anion exchanger 1 (AE1) and H^+-ATPase immunostaining. The size of AE1 (brown color) and H^+-ATPase (blue color)-positive type A intercalated cells (arrows) significantly increased in the low K^+ diet group when compared to control (a,b). Conversely, in the high K^+ dietary group, type A intercalated cells decreased in size (c).

3.3. Response of sideroblast subtypes

In the collecting duct, type A sarcoplasmic reticulum cells have an H^+-ATPase on the apical side of the cell membrane and an anion exchanger, AE1, on the basolateral side [17-19]. Neither H^+-ATPase nor AE1 is expressed in the primary cells. Image analysis showed that the size of type A cells in the low-potassium diet group increased to 161.3±19.74% compared to the control group, and decreased to 77.9±6.54% in the high-potassium diet group (Figure 2).

Type B and NANB cells have in common a separate anion exchanger, pendrin, on the apical surface of the cell membrane, which is different from AE1 [17,19,23,24]. The distinction between the two cells is based on anatomical grounds, with type B cells predominantly distributed in the collecting duct and type NANB cells restricted to a narrow region of the connecting tubule [18,19]. Image analysis showed that both type B (59.7±7.56%) and NANB (62.0±8.60%) cells in the low-potassium diet group were significantly reduced in size compared to the control group. Conversely, in the high-potassium diet, the size of type B cells (139.1±14.45%) increased.

4. Discussion

This study demonstrates for the first time that the collecting duct of the kidney can change morphologically in response to potassium intake, and that different podocyte subtypes respond differently.

Cardiovascular disease, including hypertension, is one of the most common and life-threatening chronic diseases in the modern world. Modern chronic diseases

are often driven by acquired factors, and Westernized diets are widely recognized as a major contributor [5,6,25].

Potassium is an essential electrolyte in the human body, and its intake is low in modern humans compared to prehistoric humans [2,3]. Potassium deficiency is a hallmark of the typical Western diet and is strongly associated with the development of hypertension and cardiovascular disease [5,6]. Recent studies continue to highlight the beneficial actions of potassium and recommend supplemental methods of intake for health [26]. Bananas, avocados, and kiwis are some of the most popular potassium-rich foods.

The intestine and kidneys are responsible for the intake and excretion of potassium, with the kidneys actively regulating potassium secretion through urine [1,13]. The process of potassium secretion from the kidneys has traditionally been characterized by the actions of the collecting duct principal cells [14,15]. However, the role of interstitial cells has recently been recognized. Traditionally, they have been thought to be involved in acid-base balance regulation, but their link to potassium and blood pressure has been increasingly recognized [27]. Anion exchangers expressed in some subtypes of pyknotic cells can exchange the monovalent anion bicarbonate (HCO_3^-) for chloride ions (Cl^-). Since chlorine can move in association with sodium, the secretion of acid or base can be strongly linked to the regulation of body fluids.

Recently, studies have reported that pendrin, an anion exchanger expressed in type B interstitial cells, has been implicated in the pathogenesis of hypertension [28-30]. Pendrin is located on the apical surface of the cell membrane of type B and NANB cells [19,23]. Pendrin is a different anion exchanger from AE1, which is expressed on the basolateral surface of type A sarcoplasmic reticulum, and type B and NANB cells do not have AE1 [23,24].

Immunohistochemically, type B cells are characterized by the presence of the H^+-ATPase on the bottom surface, whereas type NANB cells are characterized by the presence of the H^+-ATPase on the top surface of the cell membrane [17-19]. However, the 'original' antibodies with clear polarity have been lost and the current commercially available antibodies have unclear staining properties. Recently, the newly discovered ammonia receptor proteins, Rhbg and Rhcg,

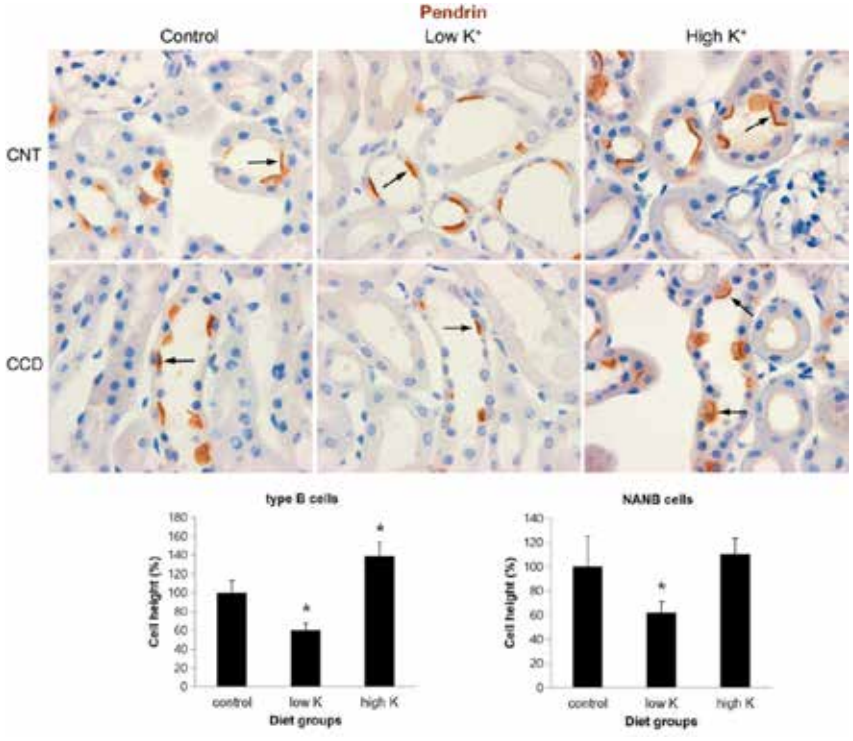

Figure 3. Pendrin immunostaining. The size of pendrin (brown color)-positive intercalated cells (arrows) significantly decreased in the low K^+ diet group in both connecting tubule (CNT) and cortical collection duct (CCD) when compared to control (a–d). Conversely, pendrin-positive cells (arrows) are enlarged in the high K^+ diet group (e,f).

which are expressed in type A and NANB cells, may be good labeling agents when used in combination with pendrin [19,31]. However, ammonia receptor-related antibodies are not yet commercially available due to limited research.

In this study, we attempted to distinguish pendrin-positive cells by the anatomical difference that B-type cells are mainly distributed in the collecting duct and NANB-type cells are only localized in the connecting tubules. Despite limitations due to the lack of markers, we were able to partially identify differences in the response of type A, B and NANB cells to potassium uptake. The responses of type B and NANB cells are particularly noteworthy and may be closely related to chloride and secondary water regulation via pendrin. These results contribute to the elucidation of the unique roles of sinoatrial cell subtypes in the pathogenesis of potassium-related hypertension and cardiovascular disease.

Disclosure statement

The author declares no conflict of interest.

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