Differential Expression of Renal Outer Medullary K\(^+\) Channel and Voltage-gated K\(^+\) Channel 7.1 in Bladder Urothelium of Patients With Interstitial Cystitis/Painful Bladder Syndrome

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OBJECTIVE
To investigate the changes including expression and localization of 2 potassium channels, renal outer medullary K\(^+\) channel (ROMK) and voltage-gated K\(^+\) channel 7.1 (KCNQ1), after increased urinary potassium leakage in patients with interstitial cystitis/painful bladder syndrome (IC/PBS).

MATERIALS AND METHODS
The study group included 24 patients with IC/PBS and a control group consisting of 12 volunteers without any IC/PBS symptoms. Bladder biopsies were taken from both groups. We determined the protein expression and distribution of potassium channels using immunoblotting, immunohistochemistry, and immunofluorescent staining under confocal laser microscopy.

RESULTS
The results revealed that ROMK was predominantly expressed in apical cells of the bladder urothelium at significantly higher levels (3.3-fold) in the study group than in the control group. In contrast, KCNQ1 was expressed in the basolateral membrane according to confocal microscopy results and did not significantly differ between groups.

CONCLUSION
Our data showed that the abundance of ROMK protein in apical cells was increased in the IC/PBS group, whereas KCNQ1, which was distributed in the basolateral membrane of the bladder urothelium, showed similar abundance between groups. These results suggest that upregulation of the ROMK channel in apical cells might permit avid potassium flux into the bladder lumen to maintain intracellular K\(^+\) homeostasis in the dysfunctional urothelium.

In interstitial cystitis/painful bladder syndrome (IC/PBS), current theories of pathogenesis include chronic or subclinical infection, autoimmunity, neurogenic inflammation, or bladder urothelial defects.\(^1,2\) In particular, high potassium levels in the urine (ie 24-133 mEq/L)\(^3,4\) can easily lead to leakage into interstitial tissue because of dysfunctional bladder urothelium, causing nerve depolarization and detrusor contractions and resulting in urgency, frequency, and bladder pain in patients with IC/PBS.\(^5,6\) Intravesical potassium chloride (0.4 M KCl, 40 mL) instillation can elicit abnormal epithelial permeability responses in the diseased bladder and is a diagnostic tool for IC/PBS.\(^8\)

Imbalance of K\(^+\) homeostasis in the bladder with IC/PBS can be modulated by certain ion transporters or channels, particularly potassium channels.\(^9,10\) Among the K\(^+\) channels in the urinary system, the renal outer medullary K\(^+\) channel (ROMK; Kir1.1) plays the central role in regulating salt and potassium homeostasis in the apical membranes of cells of the thick ascending limbs, distal and connecting tubule, and outer medullary collecting duct of the kidneys in mammals.\(^11-13\) In contrast, another K\(^+\) channel, voltage-gated K\(^+\) channel 7.1 (KCNQ1; Kv7.1), is essential for recycling potassium across the basolateral membrane of epithelial cells.\(^14\)

Previous studies found that the 2 potassium channels exist in the bladder epithelium. Although previous studies reported that ROMK and KCNQ1 are present in different locations, the present study showed that ROMK is predominantly expressed in apical cells and KCNQ1 in basolateral membrane, suggesting a different role in potassium homeostasis.
locations of mammalian bladder tissues, this is the first study to investigate protein expression of both potassium channels in the dysfunctional bladder urothelium after increased urinary potassium leakage in patients with IC/PBS. Our results increase the understanding of the molecular changes that occur in the ROMK and KCNQ1 channels in the diseased bladder.

**MATERIALS AND METHODS**

**Patient and Tissue Samples**
Bladder specimens were obtained from 24 patients (21 women and 3 men) with IC/PBS who were undergoing cystoscopy under anesthesia for the diagnosis of therapeutic bladder distention. Patients met the cystoscopic criteria established by the National Institute of Diabetes and Digestive and Kidney Diseases, including moderate to severe disease symptoms of greater than 6 months’ duration, and had an average age of 42.2 years (range: 23-57 years). In the study group, there was no mucosal disease, so a cold-cup biopsy was taken from the site showing glomerulations after bladder hydrodistention and ruled out the bladder cancer by pathologic report. Control bladder biopsy specimens were obtained from 12 patients (median age: 53.5 years, range: 42-58; 10 women and 2 men), undergoing a bladder neck suspension procedure for stress urinary incontinence, who showed no evidence of IC/PBS or bladder mucosal disease. All tissues were stored at -80°C for immunoblotting or fixed in formalin for immunostaining. All specimens were removed only after obtaining informed written consent from the patients. This study was approved by the Institutional Review Board of Kaohsiung Armed Forces General Hospital.

**Antibodies**
Four primary antibodies were used in the present study: (1) ROMK, a goat polyclonal antibody for immunoblotting and immunostaining (sc-10694; Santa Cruz Biotechnology, Santa Cruz, CA; dilution 1:500 and 1:50, respectively); (2) KCNQ1, a goat polyclonal antibody for immunoblotting and immunostaining (sc-10646; Santa Cruz Biotechnology; 1:500 and 1:50, respectively); (3) actin, a rabbit polyclonal antibody (sc-1616-R; Santa Cruz Biotechnology; 1:500 and 1:50, respectively); (2) KCNQ1, a goat polyclonal antibody (NCL-L-CK7-OVTL; Novocastra, Newcastle upon Tyne, UK; dilution 1:400) for immunostaining to identify the bladder epithelium. The secondary antibodies for immunoblotting included horse-radish peroxidase-conjugated rabbit anti-goat immunoglobulin G (305-035-003; Jackson ImmunoResearch, West Grove, PA) or goat anti-rabbit immunoglobulin G (GTX213110-01; GeneTex, Hsinchu, Taiwan). The secondary antibodies for immunofluorescent (IF) staining included the Alexa Fluor 488 conjugated donkey anti-goat and the Alexa Fluor 546 conjugated goat anti-mouse antibodies (Molecular Probes, Eugene, OR; dilution 1:100 or 1:200, respectively). Preliminary experiments of negative controls (samples stained with only primary or secondary antibodies) demonstrated neither nonspecific staining nor overstaining of the background (data not shown) in IF staining.

**Immunoblotting**
The method used for immunoblotting was modified from our previous studies. Briefly, aliquots containing 50 μg of homogenates prepared as stated previously were mixed with sample buffer and then heated at 95°C for 5 minutes. After electrophoresis and transformation, the blots (polyvinylidene fluoride membranes; Millipore, Bedford, MA) were pre-incubated for 2 hours in phosphate buffer saline buffer (137 mM NaCl, 3 mM KCl, 10 mM Na2HPO4, 2 mM KH2PO4, pH 7.4) containing 0.05% Tween 20 and 5% (wt/vol) nonfat dried milk to minimize nonspecific binding. According to the manufacturer’s manual, antibodies for ROMK, KCNQ1, and actin revealed molecular weights of approximately 50, 75, and 42 kDa, respectively. The blots were cut into upper and lower parts and then incubated sequentially in blocking buffer, primary antibody (ROMK, KCNQ1, or actin), and secondary antibody. Finally, protein bands were detected using the SuperSignal West Pico Detection Kit (#34082; Pierce, Rockford, IL) and imaged using a cooling charge-coupled device image sensor (ChemilDoc XRS+; Bio-Rad, Hercules, CA) with software (Quantity One 4.6.8; Bio-Rad). The results were converted to numerical values to compare the relative protein abundances of the immunoreactive bands.

**Immunohistochemical Staining**
Immunohistochemical (IHC) staining was conducted as previously described with some modifications. In brief, formalin-fixed and paraffin-embedded sections (4 μm) of samples were dewaxed and then rinsed by phosphate buffer saline buffer. Endogenous peroxidase was inactivated by incubating the sections with 3% hydrogen peroxide. Next, the sections were stained with an antibody (CK7, ROMK, or KCNQ1) or phosphate buffer saline buffer (negative control) before analysis using a commercial kit (PicTure; Zymed, South San Francisco, CA). Finally, the sections were counterstained with hematoxylin (#1.05175.0500; Merck, Darmstadt, Germany) and rinsed with tap water. All sections were observed by an optic microscopy (BX50; Olympus, Tokyo, Japan) with a cooling charge-coupled device (DP72; Olympus) using CellSens 1.4 software (Olympus).

**Multiple IF Staining and Confocal Laser Microscopy**
The method for IF staining was carried out as described previously. Deparaffinized sections prepared as above were incubated at 4°C overnight with the diluted primary antibodies (CK7, ROMK, or KCNQ1). Following incubation, the samples were washed and then exposed to the respective secondary antibody for 1 hour. Finally, the sections were covered with a coverslip using mounting solution (DAPI Fluoromount-G, #0100-20, SouthernBiotech, Birmingham, AL).

To determine and compare the localization of specific proteins, IF-stained sections prepared as described above were observed using a confocal laser scanning microscope (Leica, Heidelberg, Germany). The method was carried out as described previously. Briefly, the 488 nm argon-ion laser and 543 nm helium-neon laser were used to observe the fluorescence staining of Alexa-Fluor-488 and Alexa-Fluor-546, respectively, to give the appropriate excitation wavelengths. The micrographs acquired from each photomultiplier were subsequently merged so that the different-colored labels could be simultaneously visualized.

**Statistical Analysis**
Data were analyzed using the Mann-Whitney U test and P <.05 was considered statistically significant. All values are expressed as the mean ± standard error.

**RESULTS**
The protein abundance of ROMK was significantly higher (3.3-fold) in the study group than in the control group (2.44 ± 0.29 standard error).
vs 0.75 ± 0.11; \( P < .05 \) (Fig. 1), whereas there was no difference in the expression of KCNQ1 between groups (0.67 ± 0.08 vs 0.62 ± 0.09) (Fig. 1). On the other hand, ROMK and KCNQ1 were mainly distributed in the bladder urothelium as identified by CK7 (epithelial marker) using immunostainings (Figs. 2, 3). In IHC staining, most immunosignals (red) were observed in the bladder urothelium compared with the negative control (Fig. 2). The IHC results showed stronger staining of ROMK in apical cells of the study group compared with the control group (Fig. 2C,G), and KCNQ1 expression did not differ between groups (Fig. 2D,H). Furthermore, double IF staining revealed that ROMK was predominantly expressed in the apical cells of the bladder urothelium of the IC/PBS group (Fig. 3B,H) and that KCNQ1 was distributed over the entire basolateral membrane of urothelial cells in both groups (Fig. 3E,K).

**DISCUSSION**

In the present study, we demonstrated by IF staining with confocal microscopy that ROMK and KCNQ1 channels are present in the bladder urothelium of humans. In addition, the protein abundance of ROMK was significantly higher (3.3-fold) in the study group than in the control group.

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**Figure 1.** Representative immunoblots (A) and relative distribution of ROMK (B) and KCNQ1 (C) in the study and control groups. Actin was used as the loading control. The asterisk indicates a significant difference (\( P < .05 \)). KCNQ1, voltage-gated \( K^+ \) channel 7.1; ROMK, renal outer medullary \( K^+ \) channel.

**Figure 2.** Micrographs of representative sections for immunohistochemical staining in the control (A–D) and study (E–H) groups after negative control (A,E), CK7 (biomarker for epithelium; B,F), ROMK (C,G), and KCNQ1 (D,H). Scale bar, 20 \( \mu \)m; asterisks, lumen; arrows, apical regions of urothelial cells. CK7, cytokeratin 7; KCNQ1, voltage-gated \( K^+ \) channel 7.1; ROMK, renal outer medullary \( K^+ \) channel.
control group and was expressed predominantly in apical cells of the bladder urothelium. In contrast, KCNQ1 expression did not differ between groups and was present in the entire basolateral membrane of urothelial cells.

The ROMK channel is specifically localized at the apical membrane of epithelial cells and plays a key role in regulating K+ concentration between the urine and the blood in the kidney. Spector et al demonstrated that ROMK was expressed in the apical membranes of the large umbrella cells lining the bladder lumen and to a lesser extent in the cytoplasm of epithelial cells and smooth muscle cells in the rat bladder. Therefore, via passing K+ across the bladder urothelia, this potassium channel may be involved not only in regulating urothelial cell volume and osmolality but also in the dissociation of K+ leaked or diffused from urine. Based on our findings, ROMK protein expression in the apical cells of the bladder urothelium of humans is compatible with the findings of the previous animal study. Additionally, higher expression (3.3-fold) of ROMK was observed in the IC/PBS group than in the control group. These results revealed that the upregulation of apical ROMK channels might permit more potassium flux into the bladder lumen and thus maintain intracellular K+ homeostasis of the dysfunctional urothelium.

The KCNQ1 channel is expressed in several other tissues throughout the body and regulates key physiological functions. It plays a crucial role in shaping the cardiac action potential as well as in transepithelial transport to control water and salt homeostasis in several tissues. Although the KCNQ1 channel is essential for recycling potassium in several epithelial cells, the expression did not differ between the study group and the control group in this study. Furthermore, the KCNQ1 protein located over the entire basolateral membrane of urothelial cells of the human bladder was determined by confocal laser microscopic observation.

A common feature of all epithelia is the polar organization of cells, which are divided into an apical (or luminal) and a basolateral membrane. The primary driving force for transepithelial transport is the activity of the sodium-potassium pump (Na+/K+-ATPase, NKA), which not only maintains cell homeostasis by transporting 3 Na+ molecules out and 2 K+ molecules into a cell but also is the driving force for the activation of other secondary ion transporters on cell membranes. We also reported that NKA was predominantly expressed in the basolateral membrane of the bladder urothelium in humans. K+ transport across the bladder urothelium from the blood into the lumen via the apical ROMK channel in concert with the basolateral NKA is the current model in the thick ascending limb of the Henle loop in the kidney. Thus, we hypothesize that K+ is secreted by the same pathway in apical cells of the bladder urothelium (Fig. 4). Basolateral NKA actively transports K+ into cells, resulting in a high K+ microenvironment, and then potassium flux into the bladder lumen via the apical ROMK channel to maintain intracellular K+ homeostasis of the dysfunctional bladder urothelium in patients with IC/PBS. Additionally, other K+ channels (eg KCNQ1 channel) may be involved in K+ recycling to maintain ion homeostasis. In this study, increased ROMK expression in the study group indicates that more K+ molecules were transported into the urine as compensation. However, there was no difference in KCNQ1 expression between the 2 groups, indicating that the KCNQ1 channel plays a role different from ROMK as described above.

This study has several limitations. First, patients’ clinical symptoms and disease duration may have interfered with the study results. Second, a small number of cases were enrolled. Third, there is no method available to detect the actual intracellular and extracellular potassium concentrations.

Figure 3. Representative horizontal micrographs of patients of the control (A–F) and study (G–L) groups after double IF staining and observed using confocal microscopy for CK7 (biomarker for epithelium [red]; A,D,G,J), ROMK (green; B,H, E,K), and merged images showed co-localization of CK7 and ROMK or KCNQ1 (C,I or F,L, respectively). Scale bar: 20 μm; asterisks, lumen; arrows, apical regions of urothelial cells. CK7, cytokeratin 7; KCNQ1, voltage-gated K+ channel 7.1; ROMK, renal outer medullary K+ channel.
CONCLUSION

Our data showed ROMK protein expression was increased in the apical membrane of cells in the IC/PBS group, whereas KCNQ1 was distributed in the basolateral membrane of the bladder urothelium with a similar abundance between groups. This suggests that upregulation of the ROMK channel in apical cells might permit avid potassium flux into the bladder lumen to maintain intracellular K⁺ homeostasis of the dysfunctional bladder urothelium.

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References

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