EGF and HB-EGF modulate inward potassium current in human bladder urothelial cells from normal and interstitial cystitis patients

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Sun Y, Chen M, Lowentritt BH, Van Zijl PS, Koch KR, Keay S, Simard JM, Chai TC. EGF and HB-EGF modulate inward potassium current in human bladder urothelial cells from normal and interstitial cystitis patients. Am J Physiol Cell Physiol 292: C106–C114, 2007. First published July 12, 2006; doi:10.1152/ajpcell.00209.2006.—Interstitial cystitis (IC) is an idiopathic condition characterized by bladder hyperalgesia. Studies have shown cytokine and purinergic signaling abnormalities in cultured bladder urothelial cells (BUC) from IC patients. We performed single-cell electrophysiological studies in both normal and IC BUC. A strongly inward rectifying potassium current with conductance of the Kir2.1 channel was identified in normal BUC. This current was significantly reduced in IC BUC. Kir2.1 protein and mRNA were detected in both IC and normal BUC. Epidermal growth factor (EGF) caused a dose-dependent decrease in the inward potassium current in normal BUC. EGF is secreted in higher amounts by IC BUC and is known to decrease Kir2.1 conductance by phosphorylation of Kir2.1. Genistein, a nonspecific phosphorylation inhibitor, increased the inward potassium current in IC BUC and blocked the effect of EGF on normal BUC. Treatment of IC BUC with heparin-binding epidermal growth factor-like growth factor (HB-EGF), previously shown to be secreted in lower amounts by IC BUC, significantly increased inward potassium current. These data show that the inward potassium current in BUC can be modulated by EGF and HB-EGF. Changes in BUC membrane potassium conductance caused by altered levels of EGF and HB-EGF may therefore play a role in the pathophysiology of IC.

epidermal growth factor; heparin-binding epidermal growth factor-like growth factor

IN-DEPTH KNOWLEDGE of bladder sensory transduction is important because a large number of patients have idiopathic chronic hypersensory bladder complaints such as urinary urgency, urge incontinence, frequency, nocturia, and bladder pain (6, 27). There are currently two bladder hypersensory syndromes: overactive bladder (OAB) and interstitial cystitis (IC). Although there is an overlap in these conditions, IC is associated with chronic bladder pain, whereas OAB is not. Therefore, IC represents a more challenging clinical problem than OAB. Unfortunately, there are no treatments that specifically target the bladder sensory pathways. The most common treatment for many of these symptoms (urinary urgency, urge incontinence, frequency, and nocturia) is the use of oral antimuscarinics whose presumed mode of action is to block detrusor smooth muscle contractions and not the sensory pathway or urothelial signal transduction.

Bladder urothelial cells (BUC) are traditionally viewed as providing barrier function, protecting the bladder stroma from urinary constituents. However, recent data suggest that BUC possibly initiate bladder sensory signals. BUC can release putative neurotransmitters such as ATP and NO in response to stretch or pharmacological stimuli (1, 17, 28, 29). BUC also have been found to express receptors typically found on neurons, including ATP receptors, P2X3/P2X2 (30, 31), muscarinic receptors (4), the transmembrane receptor potential (TRP), and TRPM8 (26). The notion that BUC may mediate bladder sensory function is supported by the finding that epithelial cells from other organs can function as “sensors.” Specifically, skin keratinocytes have been shown to express TRPV3, which could possibly mediate the skin’s response to heat (22).

IC patients’ symptoms represent the extreme spectrum of bladder sensation. Current theories regarding the etiologies of IC include increased bladder urotheal permeability (3, 21), augmented purinergic pathway activity (28, 30, 31), and cytokine alterations (13, 14). Recently, an inhibitory cytokine termed antiproliferative factor (APF), which appears to be produced only by IC BUC, was fully characterized and was found to match the peptide sequence of a segment of the sixth transmembrane domain of frizzled-8 (15). APF also may play a role in the pathogenesis of IC, given that treatment of normal BUC with APF induced changes in cell proliferation, gene expression, tight junction formation, and growth factor [including epidermal growth factor (EGF) and heparin-binding EGF-like growth factor (HB-EGF)] production, as seen in IC BUC (16, 34). Although these theories for IC pathophysiology vary in mechanisms, an intrinsic abnormality in the BUC may play a central role in pathophysiology of IC.

Because of the potential for the BUC to participate in bladder sensory signaling, single-cell electrophysiological experiments in human normal and IC BUC were performed. An inward rectifying potassium current was detected in all cells. A decreased conductance was detected in IC BUC. Extracellular EGF reduced the inward potassium current in normal BUC. Conversely, increased inward potassium conductivity was induced in IC BUC by exposure to genistein or HB-EGF. These
findings suggest that potassium currents caused by alterations in growth factor levels could play a role in the pathophysiology of IC BUC function. Future novel therapeutic interventions in hyperalgesic bladder conditions such as IC and possibly OAB could target abnormalities in BUC membrane channel properties.

MATERIALS AND METHODS

Patient selection. The diagnosis of IC was made in patients who met National Institute of Diabetes and Digestive and Kidney Diseases criteria for IC (8). Control (normal) patients had no voiding symptoms by history and showed no glomerulations after bladder hydrodistention.

Human urothelial cell culture. The protocol to obtain cystoscopic bladder urothelial biopsies for cell culture was approved by the Institutional Review Board of University of Maryland at Baltimore. All study patients were counseled, gave informed consent, and had their bladders biopsied. The process of cell culture from cystoscopic urothelial biopsies has been described previously (32). In brief, cystoscopic cold-cup bladder urothelial biopsies were performed under regional or general anesthesia. These specimens were transported to the laboratory within 30 min. They were then minced manually into 0.5-mm pieces. The samples were placed in uncoated plastic tissue culture plates (Falcon, Franklin Lakes, NJ) containing complete cell medium composed of MEM plus L-glutamine (Mediatech, Herndon, VA) supplemented with 1 U/ml insulin, 10% heat-inactivated FBS, 1.25 g/ml amphotericin B, 100 U/ml penicillin, and 100 g/ml streptomycin (GIBCO BRL, Grand Island, NY). Samples were anchored to the bottom of the well with sterile penicillin, and 100 g/ml amphotericin B, 100 U/ml penicillin, and 100 g/ml streptomycin (GIBCO). For storage, cells were centrifuged at 2,000 g, reconstituted in complete medium including 7% DMSO, and placed quickly in liquid nitrogen.

Electrophysiology of BUC. Experiments were carried out on cultured BUC serum starved at least 12 h before the electrophysiological recording. Membrane currents were amplified (Axopatch 200A; Axon Instruments, Foster City, CA) and sampled online at 5 kHz with the use of a microcomputer equipped with a digitizing board (Digidata 1200A; Axon Instruments) and running Clampex software (version 8.0; Axon Instruments). Macroscopic currents were recorded in intact cells by using the conventional whole cell configuration. Single-channel currents were recorded in cell-attached membrane patches. Patch-clamp pipettes, pulled from borosilicate glass (Kimax; Fisher Scientific, Pittsburgh, PA), had resistances of 4–5 MΩ for single-channel recordings and 2–3 MΩ for experiments using the conventional whole cell technique. The bath electrode was an Ag-AgCl pellet (Clark Electromedical, Reading, UK) that was placed directly in the bath. Cells with seal resistances <3 GΩ and access resistances >50 MΩ were discarded. Membrane currents were measured during step pulses (200 or 600 ms) or during ramp pulses (0.38 mV/ms) from a holding potential of −80 mV. All experiments were performed at room temperature, 20–22°C. For the experiment in Fig. 1B, macroscopic currents were measured during step pulses (600 ms) from a test potential of −140 mV to 10 mV at 10-mV intervals. The outward current was the focus of Fig. 2A, so the test potential was from −70 to 80 mV.

For some experiments (Fig. 1C), we used cell-attached patches to measure single-channel currents. For these experiments, calculation of the reversal potential (Erev) of a channel requires knowledge of the actual cell membrane potential (Ecm). Experiments were carried out assuming Ecm = −67 mV. After single-channel data collection, the recording was converted from a cell-attached to a conventional whole cell configuration to measure Em. The mean value of Em measurement was used to compute the proper value of Erev, for the single-channel recordings (23).

Recording solutions. For whole cell and cell-attached recordings, we used a bath solution containing (in mM) 135 NaCl, 10 KCl, 1 CaCl2, 1.2 MgCl2, 10 HEPES, and 10 glucose, pH 7.4. The pipette solution contained (in mM) 145 KCl, 1 MgCl2, 0.2 CaCl2, 5 EGTA, and 10 HEPES, pH 7.2. The measured osmolality of the pipette solutions was ~295 mosmol/l, and that of the extracellular solution was ~300 mosmol/l (Precision Systems, Natick, MA). Tetrodotoxin, charybdoxin, and iberiotoxin were obtained from Calbiochem (San Diego, CA). 4-Aminopyridine (4-AP) was obtained from Sigma (St. Louis, MO).

Holding currents were not subtracted from recordings. Single-channel amplitudes used to calculate slope conductance were obtained by fitting a Gaussian function to an all-points amplitude histogram of records obtained at various potentials. Statistical analysis was carried out using Student’s t-test for unpaired samples (Origin 7.0; Microcal Software, Northhampton, MA). Data are given as means ± SE.

Fig. 1. Inward current properties of normal bladder urothelial cells (BUC). A: BUC exhibited a strongly inward rectifying macroscopic current-voltage (I-Vm) relationship. The reversal potential of the current (Em) was nearly equal to the K+ equilibrium potential (Ek). B: the inward current was sensitive to Ba2+, a K+ channel blocker. C: cell-attached patch recording traces, from the same patch, showed an inward rectifying pattern of single-channel activities. D: plot of single-channel current (i)-Vm relationship showed the inward rectifying channel had a conductance of 22.7 pS, n = 6.
SDS-PAGE and Western blot analysis for Kir2.1 and BK. Cultured cells were treated with 0.25% trypsin for 15 min to release them from their plates. The cells were retrieved by centrifugation and washed three times with cold PBS buffer. Rat kidney homogenate served as a positive control for inward rectifying potassium channel subfamily 2.1 (Kir2.1) current and large-conductance calcium-activated potassium channel (BK) current. Cell pellets were then lysed with ice-cold denaturing buffer and incubated on ice for 1 h. The entire cell lysate was used for this experiment. Protein quantification was determined using Bio-Rad protein assay kit according to the manufacturer’s protocol. We performed 10% SDS-PAGE with 50–70 μg of protein loaded for each sample. Once SDS-PAGE was finished, the gel was electrotransferred to polyvinylidene difluoride membranes and blotted for 1 h with 5% nonfat milk blocking solution in Tween-containing Tris-buffered saline (TBST) buffer. The membrane was incubated overnight at 4°C with 1:100 rabbit anti-human Kir2.1 antibody (Alomone Labs, Jerusalem, Israel) or 1:300 rabbit anti-mouse BK antibody (Alomone Labs) in TBST buffer containing 5% nonfat milk. The following day, the membranes were washed with TBST, incubated for 2 h at room temperature with corresponding anti-rabbit secondary antibody (1:1,000), and then visualized with enhanced chemiluminescence reagents. For molecular mass determination, SDS-PAGE was performed together with prestained standard protein markers with molecular mass range between 250 and 10 kDa. Membranes were subsequently incubated with 1:25,000 actin antibody to validate the technique.

RT-PCR and PCR for Kir2.1 transcript. RT-PCR was performed to test the mRNA level for Kir2.1 for IC and normal BUC. Total RNA from BUC were harvested using Trizol reagent (GIBCO) according to the manufacturer’s instructions. First-strand cDNA synthesis was performed using total RNA primed with oligo(dT)12–18. Superscript RT (Invitrogen, Carlsbad, CA) was used for RT-PCR. PCR reactions were performed in 50-μl final volumes containing 2 μl of cDNA, 0.5 μM of each primer, 200 μM dNTPs, 2.5 units of Taq DNA polymerase, and 1× Taq DNA polymerase reaction buffer (Promega, Madison, WI). The PCR primer sequences used for Kir2.1 transcript amplification sequence were 5'-AAGTCCACACCGACAACAGTG-3' and 5'-TTCTGGATTTGGAGGACGCTG-3' for an expected amplicon size of 600 bp. The PCR conditions used were 5 min at 95°C and 40 cycles for 1 min at 95°C, 1 min at 54°C (Kir2.1) or 60°C (glyceraldehyde-3-phosphate dehydrogenase, GAPDH), and 1 min at 72°C, with extension conditions of 10 min at 72°C.

GAPDH served as a positive control, and negative controls included amplifications performed using specific primers in the absence of cDNA and amplifications that included cDNA but not specific primers. In addition, amplification was performed on RT product obtained from pipette solution aspirate without cells. PCR products were electrophoresed on 1.2% agarose gels and stained with ethidium bromide.

Fluorescence-activated cell sorting analysis for Kir2.1. Flow cytometry techniques were used and fluorescence-activated cell sorting (FACS) analysis was performed to quantify the expression difference of Kir2.1 protein in a whole cell preparation. The technique has been used before (30). Briefly, cell pellets were collected at 105 cells per sample and washed three times using FACS buffer (containing 3% fetal calf serum in 0.01 M phosphate-buffered saline with the addition of 1g/l Na3). The samples were then incubated with rabbit anti-human Kir2.1 (1:200) for 1 h at 4°C. After three washes with FACS buffer, the samples were incubated with monoclonal FITC conjugated anti-rabbit IgG (1:20) at 4°C in the dark. After three washes with FACS buffer, they were ready for the FACS analysis. During flow cytometry, cells were analyzed with CellQuest software (Becton Dickinson, Sunnyvale, CA). For each run, 10,000 cells were collected. Signals were gated to exclude debris but include most dispersed cells. Histograms of fluorescence intensity were plotted against cell numbers (events). The percentage fluorescent density was measured.

EGF Treatment. IC and normal BUC were patch clamped, and four recordings were taken to establish a baseline conductance. Recombinant human EGF (R&D Systems, Minneapolis, MN) was then dissolved in the bath solution to a concentration of 20 ng/ml. The infusion was run over the bath plate at 5 ml/min. Recordings were taken from the beginning of the infusion every 30 s for 5–10 min.

The secondary protocol involved infusing EGF at 0.6, 2, 6, 20, and 60 ng/ml to establish a dose-response curve for normal BUC. Before infusion, the cells were patch clamped and baseline recordings were taken as described above. EGF was then infused at a concentration of 20 ng/ml as described above. Recordings were taken every 30 s for the requisite 5–10 min.

The tertiary protocol involved infusing EGF at 0.6, 2, 6, and 20, and 60 ng/ml to establish a dose-response curve for normal BUC. Before infusion, the cells were patch clamped and baseline recordings were taken as described above. The infusion rate again was 5 ml/min. Recordings were taken from the beginning of the infusion every 30 s for 5–10 min.

Genistein treatment. The electrophysiological measurements were made in the manner previously described. Once a successful patch clamp was achieved on a BUC, recordings were taken every 30 s for 2 min for a total of four for each cell. These recordings were used as a baseline. Genistein (Sigma) was then dissolved in the bath solution to a concentration of 100 μM for 10 min at 37°C. The genistein was then removed and replaced with standard bath solution. The cells were then patch clamped and a baseline recording was taken as described above. EGF was then infused at a concentration of 20 ng/ml as described above. Recordings were taken every 30 s for the requisite 5–10 min.

Statistics. Data are presented as means ± SE. Student’s t-test was used to compare the differences in the mean between IC and normal BUC.
RESULTS

Electrophysiological findings in normal BUC. We measured currents of 35 BUC from four different normal asymptomatic patients. For the majority of these cells (32/35), whole cell macroscopic currents were characterized by large inward currents at negative potentials, large outward currents at positive potentials, and a flat “plateau” region at intermediate potentials, typical of strongly rectified membrane current-voltage (I-V) curves (Fig. 1A). We measured the resting potential of normal BUC. These cells had resting potentials ($E_m = -65.7 \pm 3.7 \text{ mV}$; 12 cells) near K$^+$ equilibrium potential ($E_K = -67 \text{ mV}$ for an exterior potassium concentration of 10 mM), indicating that potassium channels determined most of the resting membrane conductance in these cells (Fig. 1A).

Inward currents negative to $E_K$ were usually 300–500 pA ($E_m = -140 \text{ mV}$). These currents had reversal potentials close to $E_K$, implying the currents were attributable to potassium channels. We applied Ba$^{2+}$, a potassium channel blocker, into the bath solution. At the concentration of 1 mM, Ba$^{2+}$ could inhibit >90% of the current, indicating that inward currents in normal BUC are chiefly composed of K$^+$-specific currents (Fig. 1B).

Because of the strongly rectified properties of the inward currents, we sought to identify the underlying inward rectifying potassium channel family members using membrane patched recording in cell-attached configuration. Original records of single-channel currents obtained during hyperpolarizing test pulses to $-40, -60, -80,$ and $-100 \text{ mV}$ (holding potential, $E_m = 0 \text{ mV}$) are shown in Fig. 1C. Amplitude histograms were constructed of events obtained at potentials from $-100$ to $-40 \text{ mV}$, and values (means ± SE) for six patches are plotted (Fig. 1D). Fit of the data indicated a slope conductance of 22.7 pS and an extrapolated reversal potential of $-0.2 \text{ mV}$, which, given 145 mM K$^+$ in the pipette, was close to the estimated K$^+$ reversal potential of 0 mV. Identification of a strongly inward rectifying $-25$-pS channel prompted us to consider that this channel might be the Kir2.1 inward rectifier channel.

At membrane potentials positive to $-40 \text{ mV}$, outward currents were observed in nearly all BUC (Fig. 2A). The size of the outward currents varied over one to two orders of magnitude between cells, from tens of picoamperes to nanoamperes.

In four cells studied, the large outward currents were partially blocked by charybdotoxin (100 nM, Fig. 2B) and iberiotoxin (100 nM, data not shown), suggesting the presence of a BK channel. Western blotting confirmed the existence of the BK channel (see Fig. 6B). The outward current that remained in the presence of charybdotoxin could be further blocked by external application of 5 mM 4-AP (Fig. 2C). Its electrophysiological and pharmacological properties suggested that this component was due to a typical delayed rectifier potassium (Kd) channel.

Altering extracellular KCl from 3 to 33 mM resulted in expected changes in the reversal potentials from $-98$ to $-37 \text{ mV}$ in a normal BUC (Fig. 3). Membrane current was unaffected by the change in extracellular potassium concentration.

Electrophysiological properties of IEC BUC. We measured the resting membrane potential of 10 IEC BUC cultured from three different patients. In contrast to the normal BUC, the IEC cells had significantly depolarized resting potentials ($E_m$ of IEC BUC = $-59.3 \pm 2.9 \text{ mV}$, $n = 10$ vs. $E_m$ of normal BUC = $-65.7 \pm 3.7 \text{ mV}$, $n = 12$; $P < 0.05$, Table 1).

The inward and outward currents in IEC BUC (Fig. 4) were both significantly decreased compared with normal BUC (Fig. 1A). The macroscopic I-V curve in IEC cells was weakly rectifying, rather than the strongly rectifying pattern in normal BUC. The differences in electrophysiological behaviors between normal and IEC cells are summarized in Table 1. Because the $E_{rec}$ in IEC has been shifted away from $E_K$ in IC BUC, this suggested a decrease in potassium conductance or increased chloride conductance.

Expression of Kir2.1 and BK in normal and IEC BUC. RT-PCR results (Fig. 5) revealed bands with the expected length (600 bp) of Kir2.1 mRNA in normal and IEC BUC. Figure 6A shows representative Western blots for Kir2.1 of BUC from three normal and three IEC patients. This analysis revealed a band with a molecular mass of ~ 60 kDa, which corresponded to the position of the Kir2.1 positive control. There appeared to be less expression of Kir2.1 in normal compared with IEC BUC. Figure 6B shows expression of BK from normal and one IEC patient.

Flow cytometric techniques were used and FACS analysis was performed to determine the expression difference of Kir2.1 protein in a whole cell preparation. Figure 7 shows representative fluorescent profiles of IEC BUC and normal BUC expression of Kir2.1 using FACS. In the absence of Kir2.1 primary antibody and FITC-conjugated secondary antibody, BUC, arrested by size, were predominantly located in the 10$^6$ to 10$^7$ area (Fig. 7A). In the absence of either FITC-conjugated secondary antibody or Kir2.1 primary antibody, the fluorescence density patterns remained the same (data not shown). When the rat basophilic leukemia cells were used as a positive control for Kir2.1 (33), higher fluorescence density was observed in M1 region (Fig. 7B). With the use of this flow cytometry correlation and the same marked region for Kir2.1 expression criteria, cells from two normal patients and three IEC patients were examined for Kir2.1 expression. Figure 7E lists the mean percentages of positive Kir2.1 fluorescence density. The differences in fluorescence density between IEC and normal BUC were not statistically significant.

EGF decreased inward potassium current in normal BUC. EGF at 20 ng/ml was infused over 12 cells from two normal patients. The response was a 91.8 ± 11.7% reduction in inward
current. Figure 8A represents the significant decrease in inward potassium current when a normal cell was exposed to 20 ng/ml EGF. Figure 8B shows EGF dose-dependent inhibition of inward potassium current conductance in normal BUC. IC cells also were subjected to the same EGF exposure protocol, but a reduction of the already small current was unable to be measured (data not presented).

Genistein was then used to pretreat three normal BUC before EGF infusion. The genistein pretreated normal BUC showed only a 22.4 ± 7.9% reduction in current during EGF (20 ng/ml) infusion (Fig. 8Cd); thus genistein pretreatment was able to block EGF’s inhibitory effect on potassium conductance.

Increases in IC BUC inward potassium current induced by the tyrosine kinase inhibitor genistein. IC BUC from two different patients and normal BUC from one patient were used to test the effect of acutely infused genistein. Recordings were taken from a total of 11 cells, 4 from each of the two IC patients and 3 from the normal patient. The inward current increased 76.4 ± 30.2% for the IC BUC compared with their baseline recordings. A representative tracing of an IC cell after treatment with genistein is shown in Fig. 9. The normal BUC showed a 20.6 ± 14.7% increase in conductance from baseline following genistein infusion. On average, <2 min of infusion time was needed to see the maximal increase in conductance for each cell. The cells were observed to return to baseline function ~3 min following the cessation of the genistein infusion.

Increases in IC BUC inward potassium current induced by HB-EGF treatment. When IC BUC were treated with HB-EGF at 20 ng/ml, increases in inward rectifying potassium current were detected that correlated with the duration of HB-EGF exposure. I-V tracings from HB-EGF-treated IC BUC are depicted in Fig. 10. A summary of the inward current density and membrane potential changes with 24- and 48-h exposure to HB-EGF is presented in Table 2. There were significant changes in both inward current density and membrane potential. These changes in IC BUC shifted its phenotype toward normal BUC (compare Table 2 with Table 1).

**DISCUSSION**

The function of the bladder is simple in theory: urine storage and emptying. Traditionally, bladder function has been investigated to understand bladder emptying because the main focus has been on regulation of bladder smooth muscle contraction. However, the interval duration that the bladder must remain quiescent and relaxed significantly exceeds the amount of time that a bladder contracts. Therefore, identification of signals that can promote bladder storage is of utmost importance. Both smooth muscle and BUC can contribute bladder relaxant signals. The concept that BUC can mediate these signals and regulate bladder function is relatively recent (1, 2, 4, 17, 26, 28–31). However, the conceptual framework in which epithelial cells lining a hollow viscous organ regulate sensation or contractility of that organ has been proposed. Unlike previous investigations of BUC, which rely primarily on animal bladders, this study examined the electrophysiological characteristics of human BUC and furthermore specifically compares the differences between normal and diseased states. If one desires to understand bladder sensory abnormalities, understanding why IC patients exhibit augmented bladder sensation seems a logical approach. Animal models of augmented bladder sensation are typically created with chemical bladder irritants such as acetic acid or cyclophosphamide. These models, although useful in probing sensory pathways, have limited applicability in human bladder hypersensation syndromes in which the

![Fig. 4](image-url)  
Fig. 4. Macroscopic current of interstitial cystitis (IC) BUC. IC BUC had reduced currents at both inward and outward directions. $E_{rev}$ of inward currents had more depolarized potential than $E_k$.  

![Fig. 5](image-url)  
Fig. 5. RT-PCR for Kir2.1 in normal and IC BUC. Lanes 1–5 (left to right): 1 and 2, Kir2.1 transcripts (600 bp); 3, DNA ladder; 4 and 5, amplified glyceraldehyde-3-phosphate dehydrogenase (GAPDH) transcripts (595 bp). Lanes 1 and 4 are normal BUC (NB); lanes 2 and 5 are IC BUC.

![Fig. 6](image-url)  
Fig. 6. Inward rectifying potassium channel subfamily 2.1 (Kir2.1) and large-conductance calcium-activated potassium channel (BK) Western blot analysis. A: Kir2.1 at the expected 60-kDa size from 3 normal (NB) and 3 IC patients. B: BK at the expected 120-kDa size. MW, molecular mass.

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<th>Characteristic</th>
<th>Normal BUC (n = 12)</th>
<th>IC BUC (n = 10)</th>
<th>P Value</th>
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<td>Membrane potential, mV</td>
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<td>-59.3 ± 2.9</td>
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<td>Outward current density, pA/pF</td>
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<td>Inward current density, pA/pF</td>
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Values are means ± SE. Significant differences exist between interstitial cystitis (IC) and normal bladder urothelial cells (BUC) in membrane potential and outward and inward current densities.
patients have not been exposed to either of these agents. There also is a feline IC model in which cats exhibit bladder symptoms similar to those of humans with IC (18).

Previously, it has been shown that BUC from IC patients have abnormalities including altered cytokine production and augmented purinergic signaling (13–15, 28, 30, 31). From these studies, it was noted that the phenotypic expression of cytokines and ATP measured in the supernatant from in vitro cultures of IC BUC correlated with the expression of these substances measured from clinical urine specimens. Since abnormalities in IC BUC persist with passage of cells in culture, which exhibit altered phenotypic expression of specific proteins, it was theorized that differences in membrane electrophysiological properties between IC and normal BUC could be measured.

Electrophysiological measurements on human BUC have not been previously reported. A strongly inward rectifying potassium current putatively mediated by Kir2.1 was detected in normal BUC. This current was diminished in IC cells, resulting in the IC cells being in a more depolarized state. Kir2.1 was expressed in both IC and normal BUC based on Western blots and FACS analyses. Interestingly, IC urine specimens have been reported to contain increased EGF, and IC BUC have been reported to produce increased EGF (13–14), and a link between EGF and Kir2.1 has been studied in other types of cells.

Fig. 7. Fluorescence-activated cell sorting (FACS) analyses for Kir2.1 in BUC. A–D show representative histograms. A: Kir2.1 negative control (primary antibody negative and secondary antibody negative). B: Kir2.1 positive control from rat basophilic leukemia (RBL) cell line. C: IC BUC. D: normal BUC. E: summary of Kir2.1 expression from 3 IC BUC and 2 normal BUC. The data represent means ± SE of the percentage of the cell population expressing Kir2.1 in triplicate samples. There was no statistical difference (P > 0.05) between IC and normal BUC % expression.

Fig. 8. EGF decreases Kir2.1 conductance in normal BUC. A: control tracing depicts average of 12 normal BUC before EGF infusion. EGF tracing shows the decrease in current following the infusion of 20 ng/ml EGF in these same 12 cells. B: dose-response curve of EGF inhibition of Kir2.1 conductance. Percent inhibition was measured for 3 cells at each EGF dose. C: step pulse recording from −80 to +80 mV. a and b show the effect on normal BUC before and after the infusion of EGF, respectively. c and d show how this effect is ameliorated by 10 min of pretreatment with genistein.
Investigators have reported that EGF can acutely suppress Kir2.1 conductance, presumably by activation of tyrosine kinase that phosphorylates Kir2.1 (33). Increased secretion of EGF by cultured IC BUC (13) could explain the differences in electrophysiological measurements in IC compared with normal BUC. To test this hypothesis, we applied exogenous EGF to normal BUC. Indeed, this caused acute inhibition of the inward potassium current in a dose-dependent fashion (Fig. 8B). The inhibitory effect of EGF could be blocked by pretreatment of the normal BUC with genistein, a nonspecific tyrosine kinase inhibitor (Fig. 8B), suggesting that phosphorylation of Kir2.1 by tyrosine kinase activity plays a role in regulating inward potassium current in human BUC. Furthermore, we could increase potassium conductance in IC BUC with exposure to 100 μM genistein (Fig. 9), thus changing an IC cell to a more normal phenotype. Genistein is nonspecific, so it may not be useful as a therapeutic agent. The relationship of a cytokine affecting ion channel function has been shown to occur with transforming growth factor-β1 (TGF-β1) and Kir2.3 in reactive astrocytes from rats (24). TGF-β1 acutely depolarized the cells through inhibition of Kir2.3. This process was not dependent on gene transcription, because the astrocytes were depolarized within minutes of exposure to TGF-β1. This type of relationship is similar to that detected when EGF was added to normal BUC. IC BUC have decreased inward current, presumably from their native overexpression of EGF; however, the signals giving rise to this are uncertain but could result from the effects of APF (13–15). Therefore, another treatment option for IC may be to block expression or activity of APF.

Suppression of HB-EGF levels in the urine of IC patients and cultured IC BUC also has been described (13, 14). We therefore tested the hypothesis that exogenously added HB-EGF can ameliorate the decreased inward potassium conductance in IC BUC. HB-EGF at 20 ng/ml, which is on the same order of magnitude detected in normal human urine specimens, increased the inward potassium current (Fig. 10). This effect was associated with duration of HB-EGF exposure (Table 2) with longer exposure to HB-EGF resulting in more normalization of the altered phenotype.

The data from this study are reminiscent of vascular endothelial cells, which have been studied electrophysiologically in...
greater detail than BUC. Many ionic conductances have been described in vascular endothelial cells (20). In the human capillary endothelial cells, the inward rectifying current (11) is similar to what was detected in this study of human BUC. In both of these cell types, there is a strongly inward rectifying current with reversal potential near $K^+$ equilibrium (Fig. 1A) that is blocked by Ba$^{2+}$ (Fig. 1B), and the current amplitude is a function of extracellular $K^+$ (Fig. 3).

The function of Kir2.1 in BUC is not known, but Kir2.1 has been proposed to function as a mechanical stress sensor in vascular endothelial cells because laminar shear stress induced one of the fastest responses in potassium conductance (10). Because the bladder is also subject to mechanical force from filling and emptying, perhaps Kir2.1 in BUC may have a similar role in regulating the bladder’s response to mechanical signals. An alteration in the conductance in Kir2.1 in IC BUC may alter the IC BUC’s ability to respond to the normal mechanical changes occurring within the bladder. Whether decreased conductance of Kir2.1 in IC BUC mediates bladder hyperalgesia is unknown at this point, but it is suggested by these results.

The vascular endothelial response to extracellular potassium also has been studied. It is known that extracellular potassium can mediate vasodilation through effects on inward rectifying potassium currents as well on release of endothelium relaxing factor (5, 7, 25). Interestingly, one of the clinical tests used to “diagnose” IC is the potassium sensitivity test in which intravascular potassium is infused into the IC patient’s bladder to determine whether there is an augmented sensory response (i.e., intense pain) (21). If patients respond to intravascular potassium (at 400 mM, which is a supraphysiological concentration even for urine) with severe pain, they are considered to have IC. Whether this pain is partly due to the observed decrease in inward potassium current in IC BUC preventing complete bladder relaxation (vis-à-vis vasodilation) is unknown. Another commonly suggested treatment for IC is to reduce urinary potassium levels, but this approach has not been validated in a controlled clinical trial.

Similar to the vasculature, the bladder urothelium has been described to release a relaxant factor. Investigators have described a urothelium-derived inhibitory factor of detrusor smooth muscle in the pig bladder (9). This factor did not appear to be nitric oxide, an adenosine nucleotide, catecholamine, GABA, or an apamin sensitive to endothelium-derived hyperpolarizing factor. Whether human BUC release this factor, whether this factor is related to the inward potassium current, and whether the amount or activity of this factor differs between IC and normal BUC are all unknown at this time.

The decreased outward potassium current detected in IC BUC was not the focus of this particular study. The data suggest that BK channels mediate the outward current (Fig. 2). Several experiments have examined the effect of BK on bladder function based on studies on BK knockout mice (19). However, in these studies, the hypothesis is that the absence of BK in bladder smooth muscle cells resulted in the urinary incontinence phenotype in these animals. The function of BK in bladder urothelial cells was not studied in these BK knockouts.

In summary, this is the first report describing the presence of an inward rectifying potassium current with a conductance of the Kir2.1 channel in cultured human BUC. In IC BUC, this current is significantly reduced with the IC BUC existing in a more depolarized state. The electrophysiological phenotype of normal and IC BUC can be switched by exposing BUC to EGF, genistein, and HB-EGF. These data suggest that these growth factors and protein phosphorylation may modulate the inward potassium current in human BUC. Furthermore, these findings showed that intrinsic abnormalities are present in membrane properties of IC BUC.

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REFERENCES


