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Gap junction channel activity in short-term cultured human detrusor myocyte cell pairs: gating and unitary conductances

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1Department of Urology, Albert Einstein College of Medicine, Bronx; 2Department of Physiology and Biophysics, State University of New York at Stony Brook, Stony Brook, New York; and 3Wake Forest Institute for Regenerative Medicine, Wake Forest University Baptist Medical Center, Winston-Salem, North Carolina

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Wang, H.-Z., Peter R. Brink, and George J. Christ. Gap junction channel activity in short-term cultured human detrusor myocyte cell pairs: gating and unitary conductances. Am J Physiol Cell Physiol 291: C1366–C1376, 2006. First published August 9, 2006; doi:10.1152/ajpcell.00027.2006.—Several independent lines of investigation indicate that intercellular communication through gap junctions modulates bladder physiology and, moreover, that altered junctional communication may contribute to detrusor overactivity. However, as far as we are aware, there are still no direct recordings of gap junction-mediated intercellular currents between human or rat detrusor myocytes. Northern and Western blot techniques were used to identify connexin expression in frozen human bladder tissue and short-term cultured human detrusor myocytes. Double whole cell patch (DWCP) recording revealed that human detrusor myocyte cell pairs were well coupled with an average junctional conductance of 6.5 ± 4.6 nS (ranging from 0.1 to 15 nS, n = 22 cell pairs). Macroscopic gap junction channel currents in human detrusor myocytes exhibited voltage dependence similar to homotypic connexin43. The normalized transjunctural conductance-voltage (G-V ≈ Gmin ≈ 0.33, V0 = 63.6 mV, Z = 0.117 or equal to 2.95 gating charges), suggestive of a bilateral voltage-gated mechanism. In symmetric 165 mM CsCl, the measured single-channel slope conductance was 26 pS for the fully open channel and 120 pS for the mostly open substate. Occasionally, subconductance states were also observed. The single-channel mean open time declined with increasing Vr, accounting for the voltage-dependent decline of macroscopic junctional current. Qualitatively similar electrophysiological characteristics were observed in DWCP of freshly isolated rat detrusor myocytes. These data confirm and extend previous observations and are consistent with reports in other smooth muscle cells types in which Cx43-mediated intercellular communication has been identified.

The function of the bladder is to fill, store, and empty urine. Specifically, the bladder must be able to fill and store urine under low pressure but still be capable of the rapid, coordinated contractions required to ensure complete emptying. All of these functions are obviously critically dependent on the tone and syncytial activation of the detrusor myocytes that comprise the bladder wall. Neural innervation and myocyte excitability (i.e., action potential generation and propagation) have traditionally been thought to be sufficient to provide the requisite mechanism. However, accumulating evidence indicates that gap junction-mediated intercellular communication among detrusor myocytes also contributes to bladder physiology and dysfunction (2, 7, 8, 20, 25, 31, 32, 36–38, 44).

For example, electrophysiological data demonstrate that current injected into one detrusor myocyte, both in situ and in culture, flows quite readily into neighboring cells (2, 20, 55). More specifically, the space constant for decremental current flow is several detrusor myocyte cell lengths (~1 mm; Ref. 20). This observation is a clear indication of the presence and potential physiological relevance of the intercellular pathway. Consistent with these data, intercellular diffusion of hydrophilic dyes (i.e., Lucifer yellow) also has been observed between detrusor myocytes in situ, and gap junction-mediated intercellular calcium waves have been documented among detrusor myocytes in culture (37, 38) and in situ (27, 28). Furthermore, electron microscopy, immunogold labeling, confocal immunofluorescence, and Western blot techniques have all confirmed the presence of small junctional plaques composed of Cx43, and also Cx45, between human detrusor myocytes (25, 32, 36, 37). Despite these functional demonstrations, others have not been able to consistently document the presence of junctional plaques (the macroscopic structural correlates of intercellular communication) between detrusor myocytes using conventional methods (11, 12, 15–18). The small size of the junctional plaques observed likely accounts for the difficulty in detecting these classic structures among detrusor myocytes in the bladder wall.

In this regard, gap junctions are well-characterized membrane specializations found in many tissues. These aqueous intercellular channels provide partial cytoplasmic continuity and mediate the movement of ions and small molecules such as second messengers, small metabolites, and, reported most recently, small interfering RNA (6, 21, 41, 47, 48), between coupled cells. Gap junction channels are formed from the union, in the extracellular space of paired hexamers (i.e., hemichannels), of subunit proteins called connexins (Cxs). The connexin family of proteins is named according to molecular weight and is encoded by at least 20 genes (22, 43), although as mentioned above, only 2 of these appear relevant to detrusor myocytes (i.e., Cx43 and Cx45). Homotypic gap junction channels formed by different connexin proteins exhibit distinct transjunctural voltage sensitivities, unitary channel conductances, and permselectivities (1, 19, 45, 50, 51, 57). These biophysical distinctions provide for electrophysiological “signatures” of the various gap junction channel proteins. As far as

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we are aware, the biophysical properties of gap junctions in human detrusor myocytes have not been reported.

A major goal of this study, therefore, was to use double whole cell patch (DWCP) recording techniques to study gap junction channel behavior in human detrusor myocyte cell pairs. These studies reveal electrophysiological characteristics consistent with the presence of a prominent population of Cx43-derived channels in detrusor myocytes. Such observations may have important implications for normal bladder physiology and bladder disease (i.e., detrusor overactivity).

**MATERIALS AND METHODS**

**Obtainment of surgical specimens.** Excised bladder tissue was obtained from five patients (4 male, 1 female), according to an Institutional Review Board protocol approved by the Committee on Clinical Investigation of the Albert Einstein College of Medicine/Montefiore Medical Center. All bladder tissue was obtained from surplus normal tissue available following removal of bladder tumors. The mean patient age was 54.6 ± 4.9 yr (range 47–74 yr).

**Cell preparation.** Homogeneous explant smooth muscle cell cultures were developed from detrusor smooth muscle strips from all five patients by using a procedure identical to that described previously for the preparation of other human smooth muscle cell types (3, 4, 6, 9, 55). Briefly, sections of detrusor muscle were placed in Dulbecco’s medium (GIBCO, Grand Island, NY) containing antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin). Tissue was washed, cut into 1- to 2-mm pieces, and placed in tissue culture dishes with sufficient nutrient medium to prevent drying. After the explants had attached to the substrate, usually within 1–2 days, more culture medium was added. When the cells had migrated from the explant and undergone division, they were detached using 0.05% trypsin and 0.02% EDTA at 37°C for 5 min. Cells were subsequently grown in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum, 2 mM glutamine, and antibiotics. Cellular homogeneity was verified by immunofluorescent staining with the use of monoclonal antibodies to human smooth muscle actin and myosin. Only passages 1–5 were used for this study. Cultures were developed from all five patients. Previous experiments have revealed that the function, expression, gross topological distribution, and other molecular biological characteristic of Cx43-derived gap junctions are conserved in cultured smooth muscle cells under these conditions (3, 4, 6, 9, 53, 55).

**Immuno staining.** Smooth muscle cells were grown in 10-mm coverslips in a 100-mm petri dish for 1–2 days (i.e., until ~60–80% confluent). At this time the medium was removed, and cells were washed three times with PBS (phosphate-buffered saline solution). Cells were permeabilized with 70% ethanol at −20°C for 10 min, thoroughly washed with PBS, and then incubated for 10 min in PBS containing 0.25% BSA before addition of the primary antibody. Cells were then incubated at room temperature for 2 h in the dark. The cells were subsequently washed in 1× PBS for 5-min intervals (a total of 4 times). The coverslips were then placed face down on slides with p-phenylene diamine/glycerol solution. Immunoreactivity was examined using a fluorescence microscope. For myosin, a mouse monoclonal primary antibody was used (Santa Cruz, Santa Cruz Biotechnology, CA; sc-6956) with a horse anti-mouse IgG secondary antibody. For human smooth muscle α-actin, the primary antibody was a mouse monoclonal antibody raised against a synthetic decapeptide corresponding to the amino-terminal portion of human smooth muscle α-actin (Sigma Chemical, St. Louis, MO), and the secondary antibody was a goat anti-rabbit IgG. Note that prior control experiments revealed no immunoreactivity in the absence of the primary actin or myosin antibody or in the presence of blocking peptide (data not shown).

**RNA preparation.** Total cellular RNA was isolated from cultured cells by using TRizol total RNA isolation reagent (Life Technolo-
gies). Briefly, cultured cells were directly lysed in culture dishes by adding TRizol reagent. The lysates from cultures were transferred to a polypropylene, round-bottom tube (Falcon, Becton Dickinson) and then incubated for 5 min at room temperature. RNA was recovered in the aqueous phase from the TRizol and chloroform mixture. RNA was precipitated in 0.1 volume of sodium acetate and 2.5 volumes of ethanol.

**Northern blot analysis for Cx43 mRNA levels in culture human detrusor myocytes.** Total RNA (20 μg) was size fractionated on a denaturing 1% agarose-formaldehyde gel and then immobilized on GeneScreen filters (NEN). The banded filters (2 h, 80°C) were hybridized in 5× SSC, 7% SDS, 10× Denhardt’s solution, 20 mM sodium phosphate (pH 7.4), and 10% dextran sulfate with 100 μg/ml denatured salmon sperm DNA and then hybridized in the same solution with the addition of a radiolabeled oligonucleotide probe of human Cx43 and human glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The blots were washed three times in 2× SSC, 0.5% SDS and subjected to autoradiography at −70°C. The sequence of the Cx43-specific probe utilized was 5’-GCAAGGGTCTACGGCAG- GCCCT-3’ (corresponding to nucleotides 952–972 in human Cx43 cDNA). The hybridization and washing temperature was 55°C. The sequence of the GAPDH probe used was 5’-AGGACGTGTGTTGGAACAGGAAT-3’ (corresponding to nucleotides 445–465 of human GAPDH, and it was used at 57°C).

**Western blot analysis for Cx43 protein levels in frozen human bladder samples.** Western blots were performed as described elsewhere (53). Frozen human bladder tissues were homogenized in 25 mM Tris–HCl buffer, pH 7.4, containing 1 mM EDTA, 2 mM DTT, and 10 μg/ml each of leupeptin, aprotinin, and PMSF and centrifuged at 800 g for 10 min. The supernatant was concentrated to a final protein concentration of ~5 μg/ml. Protein samples (30 μg each lane) were dissolved in SDS-PAGE sample buffer and loaded into 10% polyacrylamide gels. After electrophoresis, the proteins were transferred to a Zeta-Probe membrane (Bio-Rad). The membranes were then blocked overnight with 5% (wt/vol) nonfat milk in 1× PBS and probed with an anti-human Cx43 antibody (Chemicon, Temecula, CA) at a 1:1,000 dilution for 2 h. After incubation, the membranes were washed three times for 10 min each with 1× PBS and then incubated with anti-mouse secondary antibody for 1 h. After three more washes with 1× PBS (again, 10 min each), the Cx43 bands were detected with ECL reagents [Amersham (now GE Healthcare Bio-Sciences), Piscataway, NJ].

**Preparation of freshly isolated rat detrusor myocytes for DWCP.** The urinary bladder was excised from rats according to a protocol approved by the Animal Care and Use Committee of the Albert Einstein College of Medicine. Urothelium-denuded strips of detrusor smooth muscle were then enzymatically dissociated as described elsewhere (52, 54, 55) and subjected to DWCP.

**Electrophysiological recording mode and solutions.** The standard dual whole cell patch-clamp (DWCP) technique was used (49). For all DWCP experiments, the bathing solution was a cesium saline containing (in mM) 165 CsCl, 30 tetraethylammonium-Cl, 1 CaCl2, 1 NiCl2, 1 MgCl2, 2 CaCl2, 1 aminopyridine, 10 HEPES, and 0.5 ZnCl2 at a pH of 7.0. The pipette solution was a cesium saline solution identical to the bathing solution, except that the CaCl2 was reduced to 0.1 mM, 0.6 mM EGTA was added, and ZnCl2 was removed (pH 7.0). The reason for using the cesium solution for both the bath and pipette is to achieve maximum inhibition of nonjunctional channel activity, while still being able to resolve unitary junctional currents in the absence of lipophilic uncoupling agents (3, 5). The voltage protocol was generated using pCLAMP 6 (Axon Instruments), and unless otherwise stated, the experimental protocol was as follows. Initially, both cells of the pair were clamped at 0 mV, and then one cell remained clamped at 0 mV while the other cell was stepped to ±100 mV in 10-mV increments. The pulse duration was 2.5 s with 5-s intervals. For single-channel events, the transjunctional voltage ($V_τ$) ranged from 10–70 mV with a step duration of 40-s to 5 min. All
Current and voltage recordings were stored as pCLAMP 6 files and simultaneously stored on videotape using a four-channel digitizing unit and videocassette recorder for off-line analysis. Note that cell pairs with a conductance $>20$ nS, or cell pairs that lost their voltage dependence, were excluded from this study.

Analysis of junctional current data. Of note, careful examination of individual DWCP experiments revealed no obvious distinguishing features in the electrophysiological characteristics of recordings obtained from cell pairs in different patients. Therefore, the data collected and reported in the present study were considered to be derived from a homogeneous patient/cellular population. As such, we did not distinguish among cell pairs from different patients for statistical purposes. In this regard, junctional channel currents were displayed as paired whole cell currents or individual junctional current traces. All current amplitude histograms were compiled from the junctional current trace for each experiment. When junctional conductance was low enough (for example, $<0.5$ nS, 1–4 channels total), unitary gap junction channel activity was resolved. All analog signals were


Fig. 2. Molecular studies of connexin (Cx)43 expression in human detrusor myocytes. Western (A) and Northern blots (B) are representative of frozen bladder tissue and short-term cultured human detrusor myocytes, respectively, revealing the presence of Cx43. GAPDH, glyceraldehyde-3-phosphate dehydrogenase.
low-pass filtered (8-pole Bessel, LPF-30; WPI, Sarasota, FL) at 1,000 Hz and digitized at 4 kHz using a DT2801A analog-to-digital board (Data Translation, Marlboro, MA) installed in an IBM personal computer AT clone. The dead time of the recording instrumentation

Fig. 4. Voltage dependence of gap junction channels in freshly isolated rat detrusor myocytes. A: $i_j$ during a 2.5-s pulse (10-mV steps to $\pm 100$ mV) undergoes a time-dependent decay when $V_j$ exceeds $\pm 50$ mV. Each voltage pulse was followed by a 5-s recovery interval. Currents were low-pass filtered at a frequency of 1,000 Hz and digitized at 4 kHz. B: instantaneous (*) and steady-state (○) $i_j$ for the experiments shown in A are plotted as a function of $V_j$. The instantaneous $i_j$-$V_j$ relationships were approximately linear during the $\pm 100$-mV $V_j$ pulses with the slope conductances $\approx 7$ nS. As with the short-term cultured human myocytes, note that the steady-state $i_j$-$V_j$ relationship deviates from linearity above $\pm 50$ mV.

Fig. 3. Voltage dependence of gap junction channels in short-term cultured human detrusor myocytes. A: macroscopic junctional current ($i_j$) during a 2.5-s pulse (10-mV steps to $\pm 100$ mV) undergoes a time-dependent decay when transjunctional voltage ($V_j$) exceeds $\pm 50$ mV. Each voltage pulse was followed by a 5-s recovery interval. Currents were low-pass filtered at a frequency of 1,000 Hz and digitized at 4 kHz. B: instantaneous (*) and steady-state (○) $i_j$ for the experiments shown in A are plotted as a function of $V_j$. The instantaneous $i_j$-$V_j$ relationships were approximately linear during the $\pm 100$-mV $V_j$ pulses with the slope conductances $\approx 8$ nS. The steady-state $i_j$-$V_j$ relationship deviates from linearity above $\pm 50$ mV. C: ratios of steady-state to instantaneous conductance taken from 11 short-term cultured human detrusor cell pairs. Voltage protocol was identical to that described in A. Each point represents a normalized $G_j$ value at corresponding $V_j$. $G_j$ declines symmetrically in both $V_j$ directions, with the greatest decrease in $G_j$ occurring when $V_j$ exceeds $\pm 50$ mV. The solid line represents theoretical fit of the data, assuming a 2-state Boltzmann distribution (see MATERIALS AND METHODS). The Boltzmann parameters are listed in Table 1.
As illustrated in Fig. 1, we established homogeneous explant cell cultures of human detrusor myocytes. Short-term cultured cells exhibited the typical spindle-shaped appearance characteristic of smooth muscle myocytes. Isolated rat detrusor myocytes.

RESULTS

Immunostaining of cultured detrusor myocytes and molecular characterization of Cx43. As illustrated in Fig. 1, we established homogeneous explant cell cultures of human detrusor myocytes. Short-term cultured cells exhibited the typical spindle-shaped appearance characteristic of smooth muscle cells (Fig. 1A), and moreover, immunostaining of these cultures were strongly positive for human smooth muscle myosin (Fig. 1B) and actin (Fig. 1C) antibodies. In addition, Western (Fig. 2A) and Northern blots (Fig. 2B) of frozen bladder tissue and short-term cultured human detrusor myocytes, respectively, revealed the presence of Cx43. These data are consistent with prior reports documenting the presence of Cx43 in human detrusor myocytes (25, 36, 37).

Macroscopic junctional current. Macroscopic junctional conductance was evaluated in 22 human detrusor myocyte cell pairs immediately after establishment of the DWCP configuration and was monitored throughout the experiment, which lasted between 10 and 40 min. A representative recording is displayed in Fig. 3. The macroscopic junctional conductance of all human myocyte cell pairs ranged from 0.1 to 15 nS with a mean ± SE value of 6.5 ± 4.6 nS.

To evaluate \( V_j \) dependence, a standard voltage protocol was applied to human detrusor myocyte cell pairs and junctional current (\( i_j \)) was recorded as shown in Fig. 3A. The experimental data shown in Fig. 3A was obtained by stepping cell 1 from a holding potential of 0 mV to various voltages in 10-mV increments to produce \( V_j \) values ranging from −100 to +100 mV. Each \( V_j \) pulse was 2.5 s with a 5-s recovery interval. Instantaneous \( i_j \) increased linearly with \( V_j \) during the entire \( V_j \) pulse. However, the steady-state \( i_j \) clearly undergoes a time-dependent decay when \( V_j \) exceeds ±50 mV. The \( V_j \) dependence of \( i_j \) is better illustrated by plotting the instantaneous and steady-state \( i_j \) values as a function of \( V_j \) as shown in Fig. 3B. In this case, the instantaneous \( i_j-V_j \) relationship approximates a straight line with a slope conductance of 7.7 nS. The steady-state \( i_j-V_j \) relationship is linear within the \( V_j \) range of ±50 mV.
but decreases above those voltages. The $V_j$-dependent relaxation appears to be symmetrical about the origin. To assess the relationship between steady-state junctional conductance ($G_{ss}$) and voltage ($V_j$), the steady-state current ($I_{ss}$) was normalized to the instantaneous junctional current ($I_{inst}$) of each pulse ($G_{ss} = I_{ss}/I_{inst}$), and results were pooled. Because the instantaneous $i_j$-$V_j$ curves were always linear, they provided a convenient method to normalize data from cell pairs with different junctional conductances. The results from a random selection of 11 of the 22 experiments on short-term cultured human myocyte cell pairs are summarized by the data plotted in Fig. 3C. Steady-state $G_j$ decreases between $V_j$ = $\pm 40$ and $\pm 80$ mV, attaining a final value of 0.33 at $V_j$ = $\pm 100$ mV. The solid line represents the best fit of the data, assuming a two-state Boltzmann distribution (see MATERIALS AND METHODS).

For Fig. 3, $G_{max} = 1$, $G_{min} = 0.33$ for plus and minus $V_j$. $A = 0.117$, which corresponds to an equivalent gating charge of 2.9 and a $V_0 = \pm 63.6$ mV. For comparative purposes, Fig. 4 illustrates a record from a freshly isolated rat detrusor cell pair. Note the striking similarity in the macroscopic current recordings from this freshly isolated rat cell pair and that observed on short-term cultured human detrusor myocytes (Fig. 3, A and B); similar records were obtained in four other experiments on rat detrusor myocytes.

Single-channel conductance. Single gap junction channel activity was recorded in cultured human detrusor myocyte cell pairs as well as freshly isolated rat detrusor myocyte cell pairs using a symmetrical 165 mM CsCl solution (see MATERIALS AND METHODS). Again, for the purpose of comparison, Fig. 5 provides an example of single-channel currents typically observed in rat myocytes. Specifically, Fig. 5A illustrates the complimentary recordings that are the hallmark of unitary junctional currents, that is, unitary events of equal amplitude and opposite direction. In this example, the top current trace is that recorded from the stepped cell (40 mV) and the bottom current record is from the cell held at 0 mV. The average unitary conductance values for the main and subconductance states, respectively, were 90 $\pm 11$ and 25 $\pm 9.5$ pS. Figure 5B represents the amplitude histogram of the bottom trace (80 s); similar results were obtained in three other experiments. A representative example of similar unitary activity observed in short-term cultures of human detrusor myocytes is shown in Fig. 6. In this experiment, the holding potential of cell 1 was $+30$ mV, and the recording reflects the activity of cell 2 (Fig. 6A), which was

![Fig. 6. Unitary current recording in short-term cultured human detrusor myocyte cell pair. A: single-channel activity at $+30$-mV and 185-s-duration voltage step. Three distinct current levels (C, O1, and O2) were apparent and are labeled. B: all-points amplitude histogram, where the dashed line represents the pdf fit. Note the 3 distinct peaks corresponding to 0 (closed) and two open channel currents with current amplitudes of 3.8 and 0.9 pA, respectively. The channel open probability ($P_o$) calculated by fitting the histogram was 0.92 and 0.03 for the fully open channel (main state) and substate, respectively. There were a total of 33 channel events with a calculated mean open time (MOT) of 5,220 ms and a corresponding mean closed time (MCT) of 412 ms. C: single-channel current-voltage ($I$-$V$) relationship for this cell pair yields a slope conductance of 26 and 120 pS for the subconductance and fully open channel states, respectively.](http://www.ajpcell.org/cgi/content/figure/full/291/6/C1371/DC2?height=952&width=718)
held constant at 0 mV. As illustrated, the junctional current trace in cell 2 (i.e., I2) displays numerous transitions between two distinct, stable current levels and one closed state (dashed lines in trace, labeled O1, O2, and C, respectively). The duration of the record in this example is 183 s. An all-points amplitude histogram for this same segment of data is shown in Fig. 6B. The full transition from the closed to open state (C-O2) measured 3.8 pA (i.e., a corresponding unitary conductance of ~127 pS), whereas the second transition (C-O1, a subconductance state) measured 0.9 pA (equivalent conductance of ~30 pS). The all-points amplitude histogram compiled from the entire 183-s segment was fit via the methods of Manivannan et al. (33), assuming one channel and one subconducting state. The calculated channel open probability (P_o) was 0.92 and 0.03 for the fully open channel main state and substrate, respectively. In this record, there were a total of 33 channel events with an average mean open time (MOT) of 5,220 ms and a mean closed time (MCT) of 412 ms. Note that these values are similar to those reported for human corporal smooth muscle cells (3). The single-channel current-voltage (i-V) relationship is shown in Fig. 6C. The linear regression fit for the composite i-V curve shown in Fig. 6C yielded slope conductances for the substrate and main state of 26 and 120 pS, respectively. Similar results were observed from an additional 12 cultured human detrusor myocyte cell pairs. The mean slope conductance for the main state from all 13 cell pairs displaying single and multichannel data was 123 ± 3.4 pS, with a corresponding slope conductance for the substrate of ~30 pS.

**Channel gating: MOT and MCT.** Figure 7 is a plot of the log of MOT and MCT from four cell pairs in which only one or two channels were active. For convenience, the MOT values are plotted against positive Vj, and the MCT values are all plotted against negative Vj. The MOT and MCT are both voltage dependent. The corresponding regression coefficients calculated for MOT and MCT were 0.9 and 0.31, respectively. These values are comparable with a previous report on cultured human corporal smooth muscle cells (3). As illustrated, the MOT declined exponentially with the increasing Vj from an average of 5.056 mS at Vj = 20 mV to 26 mS at Vj = 70 mV. The MCT also declined with increasing Vj from an average of 2,103 ms at Vj = 20 mV to 230 ms at Vj = 60 mV. In short, MOT declined by a factor of ~250 over a Vj range of 50 mV (20–70 mV), whereas MCT declined by a factor of ~10 over a similar voltage range (20–60 mV). From the MOT and MCT data, the open probability can be calculated [where P_o = MOT/(MOT + MCT)]. Values for P_o ranged from 0.8 at 20 mV to 0.12 at 60 mV. These values are similar to those reported by Brink et al. (3) for Cx43-derived gap junction channels in human corporal myocytes.

Figure 8, A and B, illustrates another feature of gap junction channels between detrusor myocytes. Specifically, the data reveal the presence of a persistent (or long-lived) subconductance state; in this case, as recorded at ±40 mV. In Fig. 8A, a particularly long-lived subconductance state is shown with a duration of ~1.6 s. The all-points amplitude histograms for the records of Fig. 8, A and B, are shown in Fig. 8, C and D, respectively. The conductance of the substrate was ~30 pS.

**DISCUSSION**

Gap junction-mediated intercellular communication plays an essential role in the integrated regulation of growth, differentiation, and function in multicellular tissues and organisms. Perturbation of gap junction function can result in altered intercellular communication and ultimately can cause irreversible damage to the integrity of a tissue (30). With respect to the urinary bladder, the current observations confirm and extend previous work in the field (2, 20, 23, 27, 28, 32, 36–38) to provide further support for an important role of gap junction-mediated intercellular communication to bladder physiology/dysfunction.

In short, the results demonstrate that short-term cultures of human detrusor myocytes are well coupled by gap junctions with macroscopic conductance values ranging from 0.1 to 15 nS, with a mean value of ~6.5 nS. Single-channel recordings revealed an average slope conductance of ~123 pS for the main state, as well as at least one major substrate with a corresponding average conductance of ~30 pS. Several other subconductance states are discernible but are too infrequent for accurate measurement of conductance or other parameters. It should be emphasized that all of the observations on human detrusor myocytes reported in the present study were performed on short-term cultures. The reason for this is related to the necessarily small size of the biopsies obtained from human bladders (see MATERIALS AND METHODS for details). Given the small sample sizes (~100–200 mg) obtained, as well as the infrequent availability of the tissue in the first place, we have not yet been able to obtain a sufficient number of freshly isolated human detrusor myocytes for DWCP analysis. Thus we have established short-term cultures of these cells, where the myocytes can be expanded and studied as required while maintaining many of their phenotypic characteristics. Furthermore, we would point out that biophysical and electrophysiological characteristics of the junctional recordings (both macroscopic and unitary conductance values) obtained on the
short-term cultured human detrusor myocytes are virtually indistinguishable from corresponding measurements made on freshly isolated rat detrusor myocytes. Since the latter has also been shown to express Cx43 between detrusor myocytes in vivo, the implication is that short-term cultures of human detrusor myocytes are a reasonable model system for beginning to study the contribution of intercellular communication to bladder function in humans.

Further evidence for the presence of homotypic Cx43-derived gap junction channels is apparent from the fact that the major slope conductance values obtained on short-term cultured human detrusor myocyte cell pairs, A and B: unitary current recordings from the same cell pair obtained at ±40-mV holding potential during a 140-s (part of the current record is shown) voltage step. The distinct (closed and open) current levels are denoted by the dashed lines. C: all-points amplitude histogram and pdf fit composed from a 26-s segment at the −40-mV Vj step. Note the 3 distinct peaks corresponding to the 0 current (closed state) and two open channel currents of 6.2 and 1.2 pA, respectively. Po calculated from the histogram data was 0.86 and 0.11 for the fully open channel and substate, respectively. D: all-points amplitude histogram and pdf composed from a 36-s segment at the +40-mV Vj step. Again, note the 3 distinct peaks corresponding to the closed state and two open channel currents with values of 5.7 and 1.2 pA, respectively. Po calculated from the histogram data was 0.89 and 0.012 for the fully open channel and substate, respectively.

Table 1. Summary of Cx43 Boltzmann parameters and single-channel conductance values

<table>
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<tr>
<th>Cell</th>
<th>n</th>
<th>Gmax (−100 +100) A Vj, mV</th>
<th>Main State Conductance</th>
<th>Subconductance</th>
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<tr>
<td>Bladder</td>
<td>22</td>
<td>1.0 0.34 0.33 0.117 63.6</td>
<td>120pS 26pS</td>
<td></td>
</tr>
</tbody>
</table>

Values for other smooth muscle cells (SMCs) are summarized from Refs. 3, 5, 35, and 53. SV, saphenous vein; IMA, internal mammary artery; CSM, corporal smooth muscle. See text for definition of Boltzmann parameters.
main state and subconductive state) as well as the $G_j-V_j$ relationship reported in the present study are consistent with Cx43 behavior (3, 35, 40, 51, 53, 55, 56). The mean open and closed times reported in the present study, which result in an open probability near 80%, are also hallmarks of Cx43-derived gap junction channels and are similar to those biophysical characteristics previously described for Cx43 in human corporal smooth muscle, saphenous vein, and internal mammary artery (3, 35, 53). That is, the mean values for all of the Boltzmann parameters, as well as the single-channel conductance values (for both the main state and substate) reported for human detrusor myocytes, are virtually indistinguishable from the values previously reported in these same three physiologically distinct human smooth muscle cell types (see Table 1).

Together, the data indicate that Cx43 is a major functional connexin protein present in normal human bladder smooth muscle cells. In addition, our biophysical observations on the short-term cultured human detrusor myocytes are consistent with other reports on human detrusor myocytes that described the presence of Cx43 in culture, in situ, and in vivo (36, 37).

For example, as recently reviewed elsewhere (8), the space constant ($\lambda$) for decremental current flow measured in detrusor smooth muscle is $\sim$1 mm, compared with a cell length of 150–200 $\mu$m. This 5- to 10-fold difference between the space constant for passive current decay and cell length is a clear indication of the presence of an adequate intercellular pathway. Consistent with these facts, the majority of recordings reported in the present study revealed macroscopic conductance values indicative of the presence of many functional channels (i.e., tens to hundreds). Of note, isolated single cells have whole cell resistances of 500 m$\Omega$ to 1 G$\Omega$. Thus the coupling conductance reported in the present study in vitro is a reflection of a relatively high conductance path between cells and provides additional mechanistic support for previous observations in situ.

Another interesting outcome of this report is the apparent lack of biophysical evidence to support the presence of Cx45-derived gap junction channels in these cultured human cells. Thus this point warrants further explanation. In this regard, the reported Cx45 unitary conductance is 20–30 pS and the voltage dependence reported by Valiunas et al. (45) reveals voltage-dependent kinetics that are an order of magnitude slower than the channel recordings reported in the present study in human detrusor smooth muscle, or those previously found in human corporal smooth muscle or vascular smooth muscle cells from human internal mammary artery or saphenous vein. Also of note, the amplitude histograms of single-channel data with subconductance states of $\sim$30 pS that are detected might at first glance be thought to be unitary Cx45 events. However, if so, then two channels are functioning in the patch, and therefore, an additional peak would be present at the 4- to 5-pA level in Fig. 5B, reflecting independent channels, one Cx43 and one Cx45 channel; clearly, this was not the case.

So, what are the implications of Cx43-derived gap junction channels to bladder function/disease? It seems reasonable to conclude that in the normal bladder, in conjunction with neurogenic (i.e., neural innervation) and other myogenic (i.e., action potential propagation) mechanisms, gap junctions provide a “safety factor” to ensure the coordinated contraction and relaxation responses required for normal bladder emptying and filling, respectively. For example, intercellular communication may well play an important role in determining the modular autonomous activity originally reported by Coolsaet et al. (10) and more recently described by Drake and colleagues (13, 14).

In fact, recent observations in human tissue biopsies from patients with neurogenic detrusor overactivity (25), as well as in patients with urge symptoms (36), clearly demonstrate an increase in the presence of Cx43-derived gap junction channels in detrusor muscle. These human data are consistent with experimental observations in a rodent model of partial urethral outlet obstruction (7) that documented a dramatic increase of Cx43 mRNA levels associated with bladder hypertrophy and overactivity (i.e., following 6 wk of obstruction). A more acute model of obstruction (i.e., hours; Ref. 23) was also associated with an increase in Cx43 mRNA expression in detrusor smooth muscle. In addition, in the trigonal smooth muscle, there is evidence for the occurrence of gap junctions (32). Consistent with the hypothesis of Coolsaet et al. (10) and Drake and colleagues (13, 14), the increased Cx43 levels seen in the obstructed/diseased bladder may well contribute to enhanced modular autonomous activity, which in turn, could account for some aspects of detrusor overactivity, and perhaps also for the increased localized modular contractions recently reported in women with urgency, with or without urodynamically proven detrusor overactivity (see Ref. 13). Nonetheless, together, these data provide good evidence for gap junction coupling between human detrusor myocytes and, moreover, for a potentially important role for altered intercellular communication in the pathogenesis of bladder disease/dysfunction.

**Perspectives.** Our data have provided, as far as we are aware, the first direct biophysical evidence for Cx43-mediated intercellular communication between human and rat detrusor myocytes. The functional data are clearly indicative of homotypic Cx43-derived gap junction channels. As with numerous other cell types, as well as exogenous Cx43 expression systems (i.e., cells transfected with Cx43 that would otherwise be devoid of intercellular communication), the normalized $G_j-V_j$ relationships are symmetrical about $V_j$ and well described by a two-state Boltzmann relationship. The voltage-dependent decline of macroscopic junctional current was due to a $V_j$-dependent decrease of channel mean open time, which was more dramatic than the decline in mean closed time. Furthermore, the voltage-dependent decline in junctional conductance was not related to a decline in the single-channel conductance. When taken in conjunction with the extant literature, these data clearly highlight the potential importance of intercellular communication to normal human bladder physiology and are also consistent with the supposition that altered Cx43-mediated intercellular communication may contribute to bladder disease/dysfunction.

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