Increased connexin43-mediated intercellular communication in a rat model of bladder overactivity in vivo

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Christ, George J., Nancy S. Day, Michele Day, Weixin Zhao, Katarina Persson, Raj K. Pandita, and Karl-Erik Andersson. Increased connexin43-mediated intercellular communication in a rat model of bladder overactivity in vivo. Am J Physiol Regul Integr Comp Physiol 284: R1241–R1248, 2003; 10.1152/ajpregu.00030.2002.—Bladder overactivity associated with outflow obstruction is a common human condition recapitulated in the female rat by narrowing the diameter of the urethra. The goal of these studies was to evaluate the role of intercellular communication through connexin43 (Cx43)-derived gap junction channels to bladder overactivity following partial urethral outflow obstruction of 3-day to 6-wk duration. Cx43 mRNA and protein expression were barely detectable by Northern or Western blots, respectively, in the detrusor layer of normal bladders, but bands were found with both techniques after 6 wk of obstruction. Linear regression analysis of the RT-PCR data revealed a statistically significant positive correlation between the duration of obstruction (again, ranging from 3-day to 6-wk duration) and Cx43 mRNA transcript levels, such that after 6 wk of obstruction, Cx43 transcript levels were ~15-fold greater than initial control values. When taking into account the approximately fivefold increase in bladder weight over this same time frame, the absolute amount of Cx43 mRNA in the bladder apparently increased by ~75-fold. In that regard, as anticipated, and consistent with previous observations, 6 wk of obstruction was also associated with a significant increase in spontaneous bladder contractions between micturitions. The amplitude of these contractions was significantly reduced by heptanol given intravesically. Furthermore, carbachol-precontracted bladder strips from obstructed animals were more sensitive to heptanol-induced relaxation (100 μM) than their unobstructed counterparts (n = 6; P < 0.01). When bladder strips were equivalently precontracted via electrical field stimulation (EFS; 20 Hz), similar heptanol-induced relaxation responses were observed. However, the tetrodotoxin-resistant portion of the EFS-induced contraction was greater in the obstructed than in the unobstructed animals, and this portion of the contractile response was more sensitive to heptanol-induced relaxation in obstructed than unobstructed bladders (n = 7; P < 0.01). Taken together, these observations indicate that partial outlet obstruction produces an overactive bladder that may be more dependent on intercellular communication through gap junctions for modulation of contractile responses than its normal counterpart.

tissue and cellular physiology; gap junction; smooth muscle; electrical field stimulation; cystometry

URINARY INCONTINENCE is a common urogenital disease that affects millions of men and women worldwide (1, 20). There are two main forms of incontinence, stress and urge, respectively. Stress incontinence typically occurs when the bladder is unable to handle the increased intra-abdominal pressure during exercise or sudden physical activity, such as coughing and sneezing. This condition is due to sphincter abnormalities and usually is provoked by physical activity. Such incontinence occurs in women who have delivered children and in men who have undergone radical prostate cancer surgery. The second main form of incontinence is urge incontinence, which is symptomatically defined by the International Continence Society as the “complaint of involuntary leakage accompanied by or immediately preceded by urgency” (1). Urodynamically, it is equivalent with “detrusor overactivity incontinence,” that is, incontinence due to an involuntary detrusor contraction. The condition is multifactorial; it is common in patients with benign prostatic hyperplasia and outflow obstruction, but the pathogenesis is not known. When there is no defined cause, the term “idiopathic detrusor overactivity” is used. When there is an associated relevant neurological condition, such as multiple sclerosis, cerebrovascular accidents, spinal cord injuries, and Parkinson’s disease, the recommended term is “neurogenic detrusor overactivity” (1, 2, 3).

By definition, the detrusor overactivity associated with urge incontinence is the result of increased smooth muscle contractions. However, the exact physiological mechanism(s) that initiates or produces the observed overactivity is not clearly understood. Two distinct hypotheses, myogenic (7) and neurogenic (19), respectively, have been advanced, and there is currently support for both hypotheses. The goal of this report was to evaluate the possibility that intercellular communication through gap junctions might participate in the pathophysiology of detrusor overactivity, regardless of the precise etiology.

In this regard, intercellular communication among constituent smooth muscle cells is a fundamental prop-

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PROPERTY OF COORDINATED TISSUE, AND THUS, OF ORGAN FUNCTION IN MOST, IF NOT ALL, SMOOTH MUSCLE TISSUES/HOLLOW ORGANS (10, 12, 16). HOWEVER, THE POTENTIALLY IMPORTANT ROLE OF INTERCELLULAR COMMUNICATION AMONG THE DETRUSOR MYOCYTES IS A FREQUENTLY OVERLOOKED ASPECT OF BLADDER PHYSIOLOGY/FUNCTION. BRIEFLY, GAP JUNCTION PROTEINS, OR CONNESSIONS, ARE FORMED BY THE UNION, ACROSS THE EXTRACELLULAR SPACE, OF TWO IDENTICAL HEXAMERIC ASSEMBLIES OF CONNESSION PROTEINS. THE MAMMALIAN CONNESSION PROTEIN FAMILY NOW NUMBERS 16, BUT THUS FAR, CONNESSION 43 (Cx43) IS THE BEST CHARACTERIZED CONNESSION IDENTIFIED IN RAT AND HUMAN BLADDER MYOCYTES (30). THESE AQUEOUS INTERCELLULAR CHANNELS PROVIDE THE ANATOMIC SUBSTRATE FOR PARTIAL CYTOSPLASMIC CONTINUITY BETWEEN ADJACENT CELLS AND ARE FREELY PERMEABLE TO MOLECULES OF UP TO ~1000 Da. THIS INCLUDES MOST OF THE PHYSIOLOGICALLY RELEVANT SECOND MESSENGER MOLECULES (I.E., cAMP, cGMP, IP3, ETC.) AND IONS (K+, Ca2+, ETC.) FOUND IN SMOOTH MUSCLE CELLS. IN LIGHT OF THE EXTANT MOLECULAR AND BIOPHYSICAL EVIDENCE FOR THE PRESENCE OF GAP JUNCTIONS IN DETRUSOR MYOCYTES IT IS LOGICAL THAT INTERCELLULAR COMMUNICATION AMONG DETRUSOR MYOCYTES CAN PLAY AN IMPORTANT ROLE IN BLADDER FUNCTION (4, 6, 8). MOREOVER, THERE ARE NUMEROUS EXAMPLES DOCUMENTING THAT THE ROLE OF INTERCELLULAR COMMUNICATION MAY VARY IN A DEVELOPMENTAL-, TISSUE-, AND/OR DISEASE-SPECIFIC FASHION AMONG OR WITHIN MYOCYTES IN ANY GIVEN ORGAN SYSTEM.

BECAUSE THE PHYSIOLOGICAL MANIFESTATION OF DETRUSOR OVERACTIVITY IS THE SAME REGARDLESS OF WHETHER ABERRANT NEURONAL OR MYOCYTE ACTIVITY IS THE PROXIMATE CAUSE, IT IS REASONABLE TO SUSPECT THAT ALTERED INTERCELLULAR COMMUNICATION PARTICIPATES IN EITHER SCENARIO. THE GOAL OF THESE INITIAL INVESTIGATIONS, THEREFORE, WAS TO BEGIN EVALUATING THE POTENTIAL CONTRIBUTION OF Cx43-MEDIATED INTERCELLULAR COMMUNICATION TO DETRUSOR OVERACTIVITY. TO THIS END, WE USED A RAT MODEL OF PARTIAL BLADDER OUTLET OBSTRUCTION THAT RECAPITULATES MANY ASPECTS OF THE PHYSIOLOGIC MANIFESTATION OF BLADDER OVERACTIVITY. THE GOAL OF THIS INVESTIGATION WAS TO BEGIN THE ROLE OF INTERCELLULAR COMMUNICATION IN THE COMMENSURATE DETERMINATION OF THE PHYSIOLOGICALLY IMPORTANT ROLE OF INTERCELLULAR COMMUNICATION IN THE COMMENSURATE DETRUSOR OVERACTIVITY.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS AND SURGICAL METHOD OF PARTIAL URETHRAL RESTRICTION TO PRODUCE BLADDER OUTLET OBSTRUCTION. FEMALE RATS (SPRAGUE-DAWLEY, 200–250 G) WERE USED IN THESE STUDIES. THE METHOD FOR PRODUCING URETHRAL CONSTRUCTION WAS IDENTICAL TO THAT PREVIOUSLY DESCRIBED (5, 32, 35). BRIEFLY, ANESTHESIA WAS INDUCED BY THE INTRAPERITONEAL INJECTION (35 MG/KG) OF PENTOBARBITAL SODIUM (ANPRO PHARMACEUTICALS), AND ANESTHESIA WAS MAINTAINED, AS REQUIRED, BY SUBSEQUENT INJECTION OF PENTOBARBITAL SODIUM (5–10 MG/KG) EVERY 45–60 MIN.


IN VIVO CYSTOMETRIC STUDIES AND EVALUATION. EVALUATION OF BLADDER FUNCTION WAS PERFORMED AS DESCRIBED ELSEWHERE (11, 32, 35, 36). BRIEFLY, THE INDWELLING BLADDER CATHETER WAS CONNECTED TO A TWO-WAY VALVE THAT WAS, IN TURN, CONNECTED TO A PRESSURE TRANSDUCER AS WELL AS AN INFUSION PUMP. THE PRESSURE TRANSDUCER WAS CONNECTED VIA A TRANSDUCER AMPLIFIER (ETH 400 CB SCIENCES) TO A DATA-ACQUISITION BOARD (MAC LAB/8E, ADI INSTRUMENTS). REAL-TIME DISPLAY AND RECORDING OF PRESSURE MEASUREMENTS WERE PERFORMED ON A MACINTOSH COMPUTER (MAC LAB SOFTWARE V3.6, ADI INSTRUMENTS). THE PRESSURE TRANSDUCERS AND A/D BOARD WERE CALIBRATED IN CENTIMETERS OF H2O BEFORE EACH EXPERIMENT.

FOR THE CYSTOMETRIC STUDIES, THE RATE OF INFUSION WAS SET ON A PROGRAMMABLE HARVARD INFUSION PUMP (MODEL PHD 2000). NOTE THAT TO OBTAIN AN APPROXIMATELY EQUAL NUMBER OF MICTURITIONS IN THE CONTROL AND OBstructed GROUPS DURING THE CYSTOMETRY PERIOD, THE RATE OF INFUSION WAS SET AT 10 AND 20 ML/H, RESPECTIVELY. THE SALINE INFUSION WAS STARTED AND CYSTOMETRIC ACTIVITY WAS RECORDED FOLLOWING THE FIRST MICTURITION AND SUBSEQUENTLY FOR AT LEAST THREE ADDITIONAL REPRODUCIBLE MICTURITION CYCLES; AS MICTURITIONS OCCUR UP TO 20 MIN APART, AT LEAST 1–2 H OF DATA WERE RECORDED ON EACH ANIMAL BEFORE DEATH. ALL EXPERIMENTS WERE PERFORMED IN A METABOLIC CAGE TO ALLOW DETERMINATION OF THE MICTURITION VOLUME. AT THE CONCLUSION OF THE EXPERIMENT, ALL ANIMALS WERE KILLED USING INTRAPERITONEAL INJECTION OF PENTOBARBITAL SODIUM. IN SOME EXPERIMENTS, HEPTANOL (100, 500, AND 1,000 MU) WAS INSTILLED INTRAVESICALLY IN RATS WITH OUTFLOW OBSTRUCTION AND DETRUSOR OVERACTIVITY.

IN VITRO RECORDING OF MECHANICAL ACTIVITY. THE RATS WERE KILLED BY CO2 ASPHYXIATION AND THE BLADDER WAS REMOVED. SEMICIRCULAR STRIPS (1 X 2 X 5 MM) WERE PREPARED FROM THE BLADDER (TO THE STRIPS WERE TRANSFERRED TO THERMOSTATICOALLY CONTROLLED (37°C) 5-ML TISSUE BATHS CONTAINING KreBS SOLUTION (IN mM: 119 NaCl, 4.6 KCl, 1.5 CaCl2, 1.2 MgCl2, 15 NaHCO3, 1.2 NaH2PO4, 5 glucose) AERATED WITH 5% CO2-95% O2 AND MAINTAINED AT A pH OF 7.4. THE STRIPS WERE ATTACHED TO TWO HOOKS BY SILK LIGATURES. ONE HOOK WAS ATTACHED TO A FORCE

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transducer FTO3C (Grass Instrument, Quincy, MA) and the other to a movable unit, which allowed adjustment of passive tension. Isometric tension was recorded using a Grass polygraph (model 7D). A TTX-sensitive transmural stimulation of nerves was accomplished by means of two platinum electrodes placed on either side of the preparations. Stimulation of nerves was performed with a Grass S48 stimulator delivering single square wave pulses (duration 0.8 ms) at the voltage giving maximal response. The train duration was 5 s, and the stimulation interval was 120 s, and the polarity of the electrodes was shifted after each pulse by means of a polarity-changing unit (22, 31).

Experimental procedure. During an equilibration period of 45–60 min the preparations were stretched to a stable passive tension of 4 mN. After this period, each experiment was started by exposing the preparations to a K+ (124 mM) Krebs solution to verify their contractile capacity. The effect of heptanol (10–300 μM) was tested on bladder contractions induced in ice-cold buffer (pH 7.2) containing: 20 mM HEPES, 0.5 mM EDTA, 1 mM DTT, 1 mM phenylmethylsulfonyl fluoride, 1 μM peptatin A, and 2 μM leupeptin. Homogenates were centrifuged at 20,000 g at 4°C, and the supernatant was then dissected free from the underlying smooth muscle. In this fashion, uretherium-denuded detrusor smooth muscle strips were prepared from the bladder.

Preparation of detrusor smooth muscle strips for molecular studies. At each time point, rats were killed via CO2 asphyxiation according to a protocol approved by the Animal Institute Committee of the Albert Einstein College of Medicine. The urinary bladder was excised and opened, and the serosal surface of the bladder was exposed under a dissecting microscope. The bladder urothelium was then dissected free from the underlying smooth muscle. In this fashion, uretherium-denuded detrusor smooth muscle strips were prepared from the bladder.

Western blot analysis. Bladder extracts were prepared from uretherium-denuded detrusor smooth muscle homogenized in ice-cold buffer (pH 7.2) containing: 20 mM HEPES, 0.5 mM EDTA, 1 mM DTT, 1 mM phenylmethylsulfonyl fluoride, 1 μM peptatin A, and 2 μM leupeptin. Homogenates were centrifugeted at 20,000 g for 30 min at 4°C, and the obtained pellet was resuspended in homogenization buffer. Protein concentrations were determined using bovine serum albumin as a standard. The samples (35 μg protein) were suspended in Laemmli sample buffer, boiled, and subjected to SDS polyacrylamide gel electrophoresis on 7.5% gels. Proteins were transferred to polyvinylidene difluoride membranes by electoblotting and the membranes were incubated in 5% nonfat milk overnight. The membranes were incubated with mouse anti-Cx43 (1:500, Zymed Laboratories, San Francisco, CA) antibody for 2 h, followed by incubation with horseradish peroxidase-conjugated anti-mouse IgG (1:5,000, Zymed Laboratories, San Francisco, CA) and horseradish peroxidase-conjugated anti-mouse IgG (1:5,000, Amersham Life Science, Arlington Height, IL).

Construction of the competitor DNA for quantitation of rat Cx43 transcript levels. A 554-bp c-erbB DNA fragment was used as an arbitrary sequence for the competitive template (Clontech). The sequences of the competitive primers were 5’-caaggtgaaatgggcaagttccttcg-3’ (forward) and 5’-agacatagggagagagtggag-3’ (reverse). PCR amplification to obtain the competitor DNA was under the following conditions: 0.2 M each of dNTP, 2 ng of the c-erbB DNA fragment, 1 μM each of the forward and reverse composite primers, with 2.5 U Taq in PCR buffer (10 mM Tris, 50 mM KCl, and 1.5 mM MgCl2). The PCR was performed with a cycle of 50°C annealing for 45 s, 72°C extension for 45 s, and 94°C denaturing for 45 s, for a total of 35 cycles with a final extension step of 8 min. The competitor DNA obtained from the primary PCR amplification was diluted to 1:100 and subject to the secondary PCR amplification with the specific primer sets of the rat Cx43 sequences under the same amplification condition. The final concentrations of the competitive template were determined with a UV spectrophotometer.

Quantification of Cx43 RNA by the competitive PCR method. The rat bladder RNA samples were prepared with TRIzol reagent (GIBCO). The specific primer sets of the rat Cx43 sequences were 5’-caaggtgaaatgggcaagttccttcg-3’ (forward) and 5’-agacatagggagagagtggag-3’ (reverse). The first strand cDNA was performed at 42°C for 1 h and then heated at 94°C for 5 min to stop the cDNA synthesis reaction under the following incubation conditions: 1 μg RNA, 20 pmol oligo dT, 0.5 mM each of dNTP, 0.5 U of RNaseout (GIBCO), and 200 Unit of MMLV RT in 50 mM Tris·HCl, pH 8.3, 75 mM KCl, and 3 mM MgCl2 buffer in a final volume of 20 μl. One volume of the first strand cDNA was then diluted with 4 vol of DEPC-H2O to a final volume of 100 μl. Five microliters of the rat bladder cDNA obtained were amplified by PCR in the presence of various competitor DNA concentrations with the specific primer set of rat Cx43. The competitor DNA was serially diluted 10-fold from 10−2 to 10−7 amol/μl for preliminary competitive PCR amplification and twofold within this concentration range for more fine-tuned amplification. The final volume of the reaction mixture was 50 μl and the PCR conditions were the same as described above, with 35 cycles determined as the optimum for the band intensities in this study.

Data analysis. Band intensities were analyzed by ImageQuan†uant software (Molecular Dynamics/Amersham Pharma*cia). Several data sets were analyzed at each time point, and unless otherwise stated, all data were expressed as means ± SE. Linear regression analysis and other appropriate statistical analyses were performed using SigmaStat software for the PC (Version 2.0; SPSS).

RESULTS

Northern and Western blot analysis of Cx43 expression in rat bladder. Conventional Northern and Western blot analysis of Cx43 expression in rat bladder was initially used to evaluate the gross effects of partial outlet obstruction on Cx43 mRNA and protein levels, respectively, in rat bladder. As shown in Fig. 1, Northern Blot analysis revealed that the Cx43 mRNA level was low in the normal rat bladder, but it increased following partial urethral obstruction. Consistent with findings at the transcript level, Western blots also revealed a marked increase in the primary PCR.
Cx43 protein levels following 6 wk of outlet obstruction (Fig. 2). In addition, the average weight of the obstructed rat bladder at the 6-wk time point was 553 ± 33 mg (n = 6), indicating the expected presence of a significant detrusor hypertrophy.

RT-PCR evaluation of Cx43 transcript levels in rat bladder. To better gauge the relative changes in Cx43 transcript expression levels during the course of partial outlet obstruction, more quantitative analyses were conducted using RT-PCR and dosimetric analysis of the resulting bands. Figure 3 shows a representative example of such an experiment conducted on a bladder from an age-matched control rat. By interpolating the relative densities in the expanded dilutions in Fig. 3B, this method allows comparison of Cx43 transcript levels in parallel with that of a mimic transcript sequence. This experimental design nominally removes PCR artifacts, thus rendering at least a semiquantitative assessment of Cx43 transcript levels. Figure 3A shows the log-fold dilutions in the mimic transcript, and Fig. 3B shows the expanded dilutions that were conducted between the two nearest match bands in Fig. 3A. In contrast to observations in age-matched control animals, an identical procedure clearly documents an elevation in Cx43 transcript levels in representative examples of experiments conducted on samples from 2-wk (Fig. 4) and 6-wk (Fig. 5) obstructed rats. The mean values for several such experiments at the 3-day, 2-wk, 4-wk, and 6-wk time points are summarized in Table 1, and a linear regression analysis of the duration of obstruction vs. the detected Cx43 transcript levels is shown in Fig. 6. As illustrated, there was a statistically significant positive correlation (P < 0.03, R = 0.92) between the detected Cx43 transcript levels and the duration of obstruction. The overall increase appears incremental with time, and the end result is a nearly 15-fold increase over baseline transcript by 6 wk of obstruction (i.e., 657 ± 42; see Table 1).

In vitro pharmacological experiments. During the equilibration period, strips from control bladders developed spontaneous contraction activity. Heptanol was found to completely abolish these spontaneous oscillations in tone. Heptanol also induced concentration-dependent relaxations in carbachol-precontracted bladder strips (Fig. 7A). Carbachol-precontracted blad-

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der strips from obstructed animals were significantly (P < 0.01; n = 6) more sensitive to heptanol-induced relaxation at 100 μM than strips from unobstructed bladders (Fig. 7A). However, similar inhibitory responses to heptanol were found in bladder strips from obstructed and unobstructed animals when the strips were contracted by EFS at 20 Hz (Fig. 7B).

Examination of the frequency-response (2–40 Hz) relationship in the presence of TTX (1 μM) revealed a significantly greater TTX-resistant portion of EFS-induced contraction in obstructed compared with unobstructed bladders (Fig. 8A). The TTX-resistant portion of the EFS-induced bladder contractions in obstructed animals was more sensitive (P < 0.05; n = 7) to heptanol than contractions in unobstructed animals (Fig. 8B).

In vivo cystometric studies. Consistent with several previous reports, 6 wk of partial urethral outlet obstruction was associated with the appearance of marked detrusor overactivity. Heptanol, instilled intravesically at a concentration of 100 μM, did not affect the urodynamic parameters that describe the micturition reflex (n = 3; i.e., bladder capacity, micturition volume, micturition pressure, residual volume, etc; see Table 2 for description of cystometric parameters) but did, however, eliminate the observed detrusor overactivity. At a concentration of 1,000 μM heptanol, dribbling incontinence was induced (n = 3). In contrast, 500 μM (n = 6) heptanol decreased micturition pressure significantly, while increasing bladder capacity and intermicturition intervals, and reduced the amplitude of the spontaneous bladder contractions (Table 2).

DISCUSSION

The role of intercellular communication in bladder function is still considered somewhat controversial because of the apparent inability to visualize morphologically definable junctional plaques at the light and

### Table 1. Cx43 mRNA levels in control animals and at various time points postobstruction

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>3 Days</th>
<th>2 Weeks</th>
<th>4 Weeks</th>
<th>6 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Connexin43 transcript level, amol/mg RNA</td>
<td>43 ± 13</td>
<td>99 ± 33</td>
<td>119 ± 24</td>
<td>235 ± 45</td>
<td>657 ± 54</td>
</tr>
</tbody>
</table>

All data are expressed as means ± SE; n = the number of urothelium-denuded bladders obtained from the same number of distinct rats. Cx43, connexin 43.

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![Fig. 6](image1.png)  
**Fig. 6.** Linear regression analysis of the effects of duration of obstruction vs. Cx43 transcript levels. As illustrated, Cx43 transcript levels increased rather incrementally over the time course of outlet obstruction, such that a ~15-fold increase was detected at the 6-wk time point.

![Fig. 7](image2.png)  
**Fig. 7.** Effects of heptanol on carbachol (10 μM; A)-precontracted isolated bladder strips and electrical field stimulation (EFS; 20 Hz; B)-induced bladder contractions.
electron microscopy levels in normal bladders. In contrast to these anatomic findings, there is clear evidence at both the molecular and biophysical levels (21, 22, 27) that Cx43-mediated intercellular communication is present, and most likely physiologically relevant, to syncytial contractions in the rat and human bladder. The issue has been discussed in recent publications (30), but the most salient supporting data are codified below.

Briefly, the space constant (λ) for decremental current flow measured in detrusor smooth muscle is ≈1 mm, compared with a cell length of 150–200 μm. Such a 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 (9, 23–25). Although the resistance of the intercellular pathway in the bladder is clearly much larger than that observed in organs known to be well coupled (e.g., myocardium) (13, 23), the membrane resistance of the detrusor myocyte is also correspondingly greater (e.g., than a cardiac myocyte), so that overall, the Rm/Ri still favors significant intercellular current flow. Thus, the detrusor smooth muscle network should be thought of as electrically well coupled (30). Moreover, if one considers the ≈10:1 asymmetry in the ratio of the junctional conductance for cell pairs (typically on the order of 5–20 nS) relative to the whole cell conductance of the typical individual myocyte (i.e., ≈0.5–2.0 nS) (10), then it is clear that the junctional component will dominate as the preferred path for ionic movement (10). Specifically, a macroscopic junctional conductance of 5–20 nS would correspond to 50–200 gap junction channels distributed around the entire cell surface area, no doubt accounting for the difficulty in visualizing junctional plaques using light or electron microscopic techniques.

Given the absolute requirement for syncytial smooth muscle contractions to the normal function of bladder emptying (18), it would not be surprising that intercellular communication acted in concert with other intrinsic mechanisms (i.e., regenerative action potentials, relatively high innervation densities, etc.) to ensure coordinated detrusor myocyte responses (21, 26). In this regard then, the overall goal of these studies was to evaluate the hypothesis that increased intercellular communication might be responsible, at least in part, for the bladder overactivity routinely observed following partial bladder outlet obstruction of 6-wk duration. The scientific rationale for this supposition is that irrespective of the precise etiology of obstruction-induced bladder overactivity (i.e., neurogenic or myopathic), increased intercellular communication would be expected to further contribute to the “escape” from the normally quiescent bladder condition during filling (27, 29).

Table 2. Effects of intravesicle heptanol (0.5 mM; n = 6) on cystometric parameters in obstructed female rats

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Before</th>
<th>After</th>
</tr>
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<tbody>
<tr>
<td>MP (cmH2O)</td>
<td>114 ± 26</td>
<td>103 ± 26</td>
</tr>
<tr>
<td>TP (cmH2O)</td>
<td>10.6 ± 1.5</td>
<td>9.8 ± 1.7</td>
</tr>
<tr>
<td>BP (cmH2O)</td>
<td>6.3 ± 1.0</td>
<td>6.2 ± 1.1</td>
</tr>
<tr>
<td>BC (ml)</td>
<td>3.0 ± 0.4</td>
<td>3.5 ± 0.1</td>
</tr>
<tr>
<td>MV (ml)</td>
<td>2.6 ± 0.3</td>
<td>2.9 ± 0.3</td>
</tr>
<tr>
<td>RV (ml)</td>
<td>0.4 ± 0.1</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td>ICI (min)</td>
<td>6.2 ± 1.5</td>
<td>7.8 ± 1.8</td>
</tr>
<tr>
<td>FSA (min)</td>
<td>1.9 ± 0.2</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td>ASA (cmH2O)</td>
<td>2.6 ± 2.1</td>
<td>9.2 ± 0.6</td>
</tr>
</tbody>
</table>

Values are means ± SE. Comparisons are made before and after drug administration. *P < 0.05, †P < 0.01 (paired Student’s t-test). MP, micturition pressure (cmH2O); TP, threshold pressure (cmH2O); BP, basal pressure (cmH2O); BC, bladder capacity (ml); MV, micturition volume (ml); RV, residual volume (ml); ICI, intercontraction interval (min); FSA, frequency of spontaneous activity (min); ASA, amplitude of spontaneous activity (cmH2O).
**Major molecular observations.** The major molecular observations of this report are therefore that first, and not surprisingly, Cx43 transcript levels are relatively low, albeit clearly detectable in the normal rat bladder. And second, that Cx43 transcript levels are considerably elevated during the time course of bladder outlet obstruction. Perhaps more importantly, the observed increase in Cx43 transcript levels is such that by the time that bladder overactivity is observed at the 6-wk time point, the magnitude of the increase is expected (∼15-fold), based on both biophysical and theoretical grounds, to be in the physiologically relevant range (14, 15, 37). More specifically, extant biophysical data and theoretical calculations indicate that ∼10-fold changes in the magnitude of intercellular communication would be required for one to observe physiologically relevant changes in intercellular communication. In fact, changes near this order of magnitude (∼5-fold increases in Cx43 mRNA levels have been reported) (33) are already suspected to be relevant and critical to the syncytial myometrial contractions observed to be coincident with parturition. Thus, the ∼15-fold increases in Cx43 transcript levels in the 6-wk obstructed bladder are certainly quite likely to be relevant to the observed hyperactivity at that same time point (15). Consistent with this observation, another recent report documented an approximately fourfold increase in detrusor smooth muscle Cx43 levels following acute (i.e., 7–9 h) outlet obstruction (28).

Furthermore, if one takes into account the approximately fivefold increase in bladder weight that occurs at the 6-wk time point, then the absolute increase in Cx43 mRNA levels in the detrusor is ∼75-fold [15-fold increase in Cx43 levels (expressed as amol/µg RNA) multiplied by the 5-fold increase in bladder weight]. Note that this calculation assumes that the fractional increase in total RNA parallels the increase in total bladder weight. In our experience, the amount of RNA isolated from urothelial-denuded bladder strips is routinely ∼1/1,000 of the total tissue wet weight.

**Major physiological observations.** The augmented Cx43 mRNA and protein levels apparently play an important role in the muscarinic receptor-mediated contraction of the obstructed bladders, as reflected by the increased heptanol sensitivity of the carbachol-induced contractile response in bladder strips from obstructed animals. The TTX-resistant portion of the contractile response elicited by EFS of isolated tissue strips, which is supposed to represent direct stimulation of the smooth muscle cell membrane, was significantly greater in bladders from obstructed animals. This is in agreement with previous studies (17, 31, 32, 34, 38) and suggests that hypertrophic bladder muscle is more sensitive to some depolarizing impulses than normal muscle (39). Interestingly, the TTX-resistant portion of the EFS-induced contractile response in obstructed animals was more sensitive to heptanol than the corresponding responses observed in age-matched control animals. Moreover, intravesically instilled heptanol (500 μM) decreased micturition pressure, increased bladder capacity and the intermicturition intervals, and decreased the amplitude of the spontaneous detrusor contractions. These in vivo findings, which are in agreement with the in vitro data, suggest that interference with intercellular communication slows down bladder activation, and ultimately stops it resulting in dribbling incontinence, which was also observed at the highest heptanol concentration used (1 mM).

In conclusion, the present data provide evidence for functional correlates of increased Cx43 mRNA and protein expression in the overactive rat bladder and indicate that the overactive bladder is apparently more dependent on intercellular communication through gap junctions for the development of at least some types of agonist-induced, myogenically mediated, contractile responses. However, it should be pointed out that our interpretation of the contribution of gap junctions to the pathophysiology of the obstructed bladder is dependent on the relative selectivity of the uncoupling actions of heptanol. In this regard, the evidence that heptanol concentrations <1 mM have predominantly junctional (i.e., gap junctional) rather than nonjunctional (i.e., ion channels such as potassium channels) effects has recently been reviewed (12). In fact, unpublished patch clamp data from our laboratory (in voltage clamp mode) indicate that the same concentrations of heptanol used in these studies (i.e., 100, 500, and 1,000 μM) have no detectable effect on whole cell outward or inward currents in freshly isolated myocytes from rat bladder (Wang and Christ, unpublished observations). When taken together then, these data provide further evidence for the development of a complex set of myogenic and tissue alterations in response to bladder outlet obstruction.

**Perspectives**

Previous studies documented that Cx43-derived gap junction channels have characteristically high unitary conductance values (∼100 pS), as well as long mean open times (1–5 s) and high open probabilities (∼90%). The main implication of these biophysical findings in tissues that contain morphologically identifiable gap junction plaques formed of Cx43 is that downregulation (i.e., decreases in intercellular communication) is the most likely scenario by which altered intercellular communication might contribute to differential tissue function. In contrast, the overactive bladder and perhaps the myometrium at parturition as well represent two examples of how increases in intercellular communication, in normally quiescent tissues, are associated with pathophysiological and physiological regulatory mechanisms, respectively, although in one sense, the increased Cx43 transcript levels could also represent a compensatory response of the bladder to the numerous physiological insults associated with bladder outlet obstruction; that is, an attempt to maintain syncytial detrusor responses in the presence of significant histopathology.

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REFERENCES


