Bladder injection of “naked” hSlo/pcDNA3 ameliorates detrusor hyperactivity in obstructed rats in vivo

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ABNORMAL BLADDER FUNCTION resulting in urinary incontinence is an extremely common problem affecting millions of men and women in the United States alone. Although both age and disease (e.g., benign prostatic hyperplasia, stroke, and diabetes mellitus) contribute to the multifactorial nature of urinary incontinence, bladder overactivity, or involuntary, uncontrolled, spontaneous detrusor contractions, leading to urge incontinence is a particularly prominent manifestation (1, 2). The mechanistic basis for increased detrusor contractility is not fully understood, but the general importance of ion channel activity to bladder smooth muscle function is well established (1, 2, 6, 25, 27). Moreover, there is evidence in the published literature for both neurogenic-based (2, 11, 19) and myogenic-based (5, 7, 17, 26, 41, 45, 51, 54) etiologies of bladder overactivity. Of note, regardless of whether the increased detrusor contractility is the product of altered neural activity or, in contrast, reflects increased myocyte contractility, or some combination thereof, the physiological result is the same, namely, increased and uncontrolled bladder contractions leading to urinary incontinence.

In this regard, a growing experimental literature indicates that altered electrical properties of detrusor myocytes (e.g., K− and/or Ca2+ channels) may contribute to the etiology of urge incontinence/bladder overactivity (7, 17, 26, 41, 51, 54). In particular, the participation of K channels in bladder overactivity has been suggested (1, 6, 26). Among the numerous K channel subtypes identified to date, the large-conductance, calcium-sensitive K channel ( maxi-K or KCa; Refs. 32, 34, 48, 50) and the metabolically regulated K channel (KATP) subtypes (4, 8, 30, 31, 46, 53, 54) are the best studied and, furthermore, may be the most physiologically relevant to detrusor myocyte function.

As reviewed elsewhere, the KCa (or maxi-K channel) plays a pivotal role in modulating contraction and relaxation responses in physiologically diverse myocytes (3, 34). Consistent with the physiological importance and therapeutic potential of the KCa channel subtype, a recent study documented that low-efficiency gene transfer of the KCa channel (i.e., hSlo, the α- or pore-forming subunit of the human large-conductance, calcium-sensitive maxi-K channel; Ref. 40) to corporal myocytes in vivo was associated with amelioration of the normally observed age-related decline in the magnitude of the cavernous nerve-stimulated intracavernous pressure response (15). This physiologically relevant effect was achieved after a single intracavernous injection of “na-
marked DNA (i.e., hSlo/pcDNA), and the clinical implication of these initial observations was that K channel gene therapy may be an option for the treatment of smooth muscle-related disorders occurring in isolated organ systems, where naked DNA can be effectively delivered in nonviral vectors, vastly diminishing the possibility of systemic complication (15).

The goal of the current investigation, therefore, was to determine if bladder instilled gene therapy with hSlo/pcDNA would also be effective in diminishing the bladder hyperactivity observed in a well-established rat model of partial urinary outlet obstruction in vivo. In fact, the dramatic bladder hypertrophy and detrusor overactivity associated with this in vivo rat model recapitulates many relevant aspects of human lower urinary tract symptoms (5, 11, 37, 42) and thus provides an excellent opportunity to further explore the utility of K channel gene therapy for the treatment of this condition and other smooth muscle disorders.

MATERIALS AND METHODS

Experimental Animals and Surgical Method of Partial Urethral Restriction To Produce Bladder Outlet Obstruction

Female Sprague-Dawley rats (200–250 g) were used in these studies. The method for producing urethral constriction was identical to that previously described (39, 42, 44). 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 (5–10 mg/kg) every 45–60 min.

After anesthetic induction, the ventral abdominal wall and perineum were shaved with an electric shaver and cleaned with betadine. A lower midline abdominal incision was made, and the bladder and the proximal urethra were identified. A plastic rod with a ~1-mm outer diameter was placed parallel to the urethra, and a silk ligature was tied/placed around the urethra and the plastic rod. After the ligature was secured, the externally dwelling plastic rod was removed by sliding the rod out from beneath the ligature, thus ensuring that the lumen diameter was constrained to ~1 mm. Animals were given the analgesic buprenorphine (0.02 mg/kg), placed under a warm lamp, and trimethoprim (4.8 mg/kg) subcutaneously, as previously described (39). The abdominal incision was then sutured closed, and the free end of the catheter was sealed. Cystometrograms were performed on unanesthetized, unrestrained rats 3 days after the surgery, as this has been shown to be an optimal time period for recovery and subsequent investigation.

Cystometric Studies and Evaluation

Evaluation of bladder function was as described elsewhere (39, 42, 44). 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 (MacLab/8e, ADI Instruments). Real-time display and recording of pressure measurements was performed on a Macintosh computer (MacLab software, version 3.4, ADI Instruments). The pressure transducers and analog-to-digital board were calibrated (in cmH2O) before each experiment.

For the cystometric studies, the rate of infusion was set on a programmable Harvard infusion pump (model PHD 2000). To obtain an approximately equal number of micturitions in the control and hypertrophic groups (both treated and untreated with hSlo) during the cystometry period, the rate of infusion was set at 10 and 20 ml/h, respectively. Cystometric activity was continuously recorded after the first micturition and subsequently for at least three additional reproducible micturition cycles; as micturitions occur ~20 min apart, at least 1.5 h of data were recorded on each animal. Experiments were performed in a metabolic cage to allow determination of the micturition volume. At the conclusion of the experiment, rats were killed by an intraperitoneal injection of pentobarbital.

Evaluation of Bladder Function

Bladder function was evaluated by the following urodynamic criteria: 1) bladder capacity, the volume of infused saline at micturition; 2) basal pressure, the lowest bladder pressure recorded during cystometry; 3) threshold pressure, the bladder pressure immediately before micturition; 4) micturition pressure, the peak bladder pressure during micturition; 5) micturition volume, the volume of urine discharged during micturition; 6) residual volume, the volume of infused saline minus the micturition volume; and 7) mean intermicturition oscillatory pressure (MIOP = IP – BP), an approximate index of spontaneous bladder contraction between micturitions. The MIOP was derived as follows. First, the average bladder pressure recorded between micturitions (intermicturition pressure) was obtained for the entire intermicturition period on each animal. The measured basal pressure (BP; see criterion 2 above) was then subtracted from the average intermicturition pressure (IP; determined as the mean pressure recorded during the entire intermicturition interval, i.e., the estimate of several thousand points, obtained using the MacLab software package) on that same animal to derive the MIOP. As such, the MIOP serves as an approximate index of the fluctuations in bladder pressure, if any, between the recorded micturition reflexes.
Evaluating Gene Transfer of Naked DNA Using the Reporter Gene LacZ Construct

pCMVβ (65 μg) or pcDNA3 plasmid (100 μg; Clontech, CA) [containing the LacZ gene under the control of the cytomegalovirus (CMV) promoter] in 1 ml PBS (containing 20% sucrose) was injected with a 28-gauge insulin syringe into the bladder lumen of a retired breeder Sprague-Dawley rat (under anesthesia during insertion of catheter; see above). Four days later the bladder was resected and stained for β-galactosidase activity. Briefly, the bladder was fixed with 4% paraformaldehyde and 0.1% glutaraldehyde for 3 h and stained with X-Gal for 12–18 h at 37°C (52). Bladders were subsequently frozen and cut into 30-μm-thick sections and placed on slides with coverslips and Permount for light microscopy. Pictures were taken with a SPOT color camera and stored on an IBM-compatible computer in the TIFF format. Images were then uploaded to Adobe Photoshop and printed out.

Preparation and Injection of hSlo/pcDNA3 into Rat Bladder

The human maxi-K channel cDNA hSlo (3.9 kb) was inserted into the Xho I-Xba I cloning site of the pcDNA3 vector (Invitrogen, Carlsbad, CA), where transgene expression is driven off by CMV promoter (15). In all cases, the amount of hSlo/pcDNA3 plasmid injected into the bladder was 100 μg in 1 ml final volume.

Detection of Steady-State Levels of hSlo Transcript in Rat Bladders

Bladders were harvested, the urothelium was quickly removed, and tissue was flash frozen in liquid nitrogen. Total RNA was extracted from frozen bladders by TRIzol reagent (GIBCO, Grand Island, NY), as described (12). RT-PCR was performed with oligonucleotide primers specific to the first six amino acids of hSlo (3′-primer: 5′-GCGGCCCACCATTT-GGCAT-3′) and T7 promoter of plasmid sequences (5′-primer: 5′-CCCTATAGAGAGTCGTATTA-3′) as described (15). The DNA sequences between the T7 promoter and the Xenopus laevis β-globin 5′-untranslated region (5′-UTR) correspond to the poly linker of the pcDNA3 vector. The X. laevis β-globin 5′-UTR is present to enhance the expression level of hSlo. The hSlo transcripts were confirmed by transblotting the RT-PCR products onto nylon membranes followed by ethidium bromide detection. The use of these primers revealed an expected 229-bp DNA fragment. The identities of the 229-bp RT-PCR products and the RT-PCR fragments were further confirmed by DNA sequencing in our Albert Einstein College of Medicine Core Sequencing Facility.

Northern Blot Analysis for Endogenous RSlo

Total RNA was extracted from rat bladders as described above. Twenty micrograms of RNA from each sample were electrophoresed in 1% agarose containing 2.2 M formaldehyde and transferred onto nylon membranes by capillary blotting. The positions of the 28S and 18S rRNA bands on the ethidium-stained gels were observed under ultraviolet illumination before transblotting. RNA was then fixed to the

![Fig. 1. LacZ staining of whole bladder. Illustration of the expression of β-galactosidase activity after a single intravesicular injection of 65 μg of LacZ/pCMVβ in the rat bladder (dissolved in 1 ml PBS) and incubation in chromogenic substrate for 18 h. A: the LacZ-injected bladder. B: for comparative purposes, a normal rat bladder. C and D: effects of 12- to 15-h incubation in chromogenic substrate on PBS-injected bladders. E–H: 4 different LacZ/pcDNA3-injected bladders (i.e., 100 μg) that were subsequently incubated in chromogenic substrate for 12–15 h and then sectioned to yield the results shown in Fig. 2.](http://ajpregu.physiology.org/10.1152/ajpregu.00749.2001)
filter by ultraviolet irradiation at 254 nm. Hybridization was carried out in Rapid-hyb buffer (Amersham, Arlington Heights, IL) at 42°C for 2 h. Membranes were washed once in 2.5× standard saline citrate (SSC) and 0.1% SDS at room temperature, followed by two washes in 1× SSC and 0.1% SDS at room temperature and 42°C, respectively. Membranes then underwent detection steps using streptavidin and biotin alkaline phosphatase with CDP-Star substrate according to manufacturer’s instruction. After CDP-Star substrate incubation, membranes were removed and exposed to the Hyperfilm (Amersham) in an intensifying screen.

RESULTS

Transgene (LacZ) Is Readily Expressed in Rat Bladder

As shown in the representative example displayed in Fig. 1, a single intravesicular injection of LacZ resulted in quite dramatic β-galactosidase activity in the bladder. Figure 1A shows the intense blue staining observed in a LacZ-injected bladder after an overnight incubation (~18 h) in chromogenic substrate, along with a normal rat bladder (Fig. 1B). Figure 1, C–H, shows bladders incubated for ~12 h in chromogenic substrate after injection of PBS (Fig. 1, C and D) or LacZ/pcDNA3 (Fig. 1, E–H). These data represent a clear demonstration that naked DNA is readily incorporated in bladder wall cells.

Histochemistry of LacZ Staining

As shown in Fig. 2, sectioning of the LacZ-injected bladders displayed in Fig. 1, E–H, revealed demonstrable punctate blue staining of the muscle layer of the bladder wall. Interestingly, the staining of the detrusor myocytes was rather heterogeneous or patchy, with pockets of fairly intense staining interspersed with regions of little or no detectable staining. In contrast, there was a rather striking and, furthermore, more uniform staining of the urothelium of the bladder of LacZ-injected rats. However, there was no detectable blue staining observed in PBS-injected rat bladders (data not shown).

Effects of Bladder Outlet Obstruction on Bladder Function In Vivo

As shown in the representative example of a cystometrogram displayed in Fig. 3, partial urethral (outlet) obstruction of 6-wk duration results in a marked alteration in bladder function compared with the micturition reflex observed in normal animals. As illustrated, the hallmark of the overactive bladder is the spontaneous bladder contractions that occur between micturitions. However, as shown in Fig. 3 and summarized in Table 1, virtually every other micturition parameter estimate was also altered after 6 wk of partial urethral obstruction. Statistical comparisons of mean parameter estimates for all cystometric data are summarized in Table 1.

Single Bladder Injection of hSlo/pcDNA Ameliorates the Bladder Hyperactivity Produced by 6 wk of Partial Urethral Constriction

Of the 22 partially obstructed Sprague-Dawley rats, 12 received a single bladder injection of naked hSlo/pcDNA (100 μg) during removal of urethral ligature...
(see MATERIALS AND METHODS). Of the 10 remaining animals, 7 received a 1-ml injection of the vehicle only (PBS), and the 3 others received an injection of 1,000 μg of the pcDNA3 vector alone. Since there were no detectable differences in the cystometrograms between the PBS alone and pcDNA3/PBS-injected animals, they were considered to represent a homogeneous group for the purposes of statistical analysis. A comparison of the recordings displayed in Fig. 3 with those in Figs. 4 and 5 clearly illustrates this point. As shown in the two distinct representative records displayed in Figs. 4 and 5, relative to the untreated, obstructed rats, the hSlo/hpcDNA3-injected rats exhibited a nearly complete ablation of overactivity (contractions or pressure fluctuations between micturitions). However, hSlo/hpcDNA3-injected rats were quite similar to the PBS-injected rats with respect to most other micturition parameter estimates. The only other significant difference in micturition parameters between obstructed rats injected with hSlo/hpcDNA3 and those injected with PBS was that the residual volume was significantly greater in the former; the residual volume in the hSlo/hpcDNA3-injected rats was also significantly greater than the corresponding value in the control (unobstructed) rats (see Table 1).

To further clarify and quantitate the effects of hSlo/hpcDNA3 injection on bladder function, we used a novel parameter estimate referred to as the MIOP. To determine MIOP, we subtracted the basal bladder pressure on each animal from the average pressure recorded in the interval between micturitions (see MATERIALS AND METHODS) on the same animal. Thus an elevation in MIOP is indicative of significantly increased bladder activity between micturitions, i.e., bladder overactivity or spontaneous bladder contractions. A comparison of the recordings displayed in Figs. 3–5 and a review of the data and statistics shown in Table 1 clearly documents the physiological relevance of the novel parameter estimate, MIOP.

Table 1. Micturition reflex parameters in age-matched control and 6-wk obstructed rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sham operated (n = 17)</th>
<th>Obstructed: hSlo injecteda (n = 12)</th>
<th>Obstructed: saline injecteda (n = 10)</th>
<th>MIOP (IP–BP), cmH2O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bladder Wt, mg</td>
<td>171 ± 15.0</td>
<td>547.6 ± 55.4‡</td>
<td>473.1 ± 56.6‡</td>
<td>3.49 ± 0.79</td>
</tr>
<tr>
<td>MP, cmH2O</td>
<td>73.9 ± 4.99</td>
<td>128.9 ± 16.1‡</td>
<td>132.7 ± 17.9‡</td>
<td>0.04 ± 3.49</td>
</tr>
<tr>
<td>THP, cmH2O</td>
<td>22.3 ± 2.1</td>
<td>36.3 ± 4.30‡</td>
<td>39.3 ± 3.6‡</td>
<td>0.04 ± 3.49</td>
</tr>
<tr>
<td>BP, cmH2O</td>
<td>12.6 ± 1.09</td>
<td>22.1 ± 4.39‡</td>
<td>18.8 ± 1.9</td>
<td>0.04 ± 3.49</td>
</tr>
<tr>
<td>BC, ml</td>
<td>1.2 ± 0.1</td>
<td>3.44 ± 0.41‡</td>
<td>2.91 ± 0.62‡</td>
<td>0.04 ± 3.49</td>
</tr>
<tr>
<td>MV, ml</td>
<td>1.13 ± 0.10</td>
<td>3.22 ± 0.39‡</td>
<td>2.94 ± 0.65‡</td>
<td>0.04 ± 3.49</td>
</tr>
<tr>
<td>RV, ml</td>
<td>0.13 ± 0.04</td>
<td>0.33 ± 0.10‡§</td>
<td>0.09 ± 0.05</td>
<td>0.04 ± 3.49</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of rats/group. Control, sham-operated, unobstructed age-matched control animals; MP, micturition pressure; THP, threshold pressure; BP, basal pressure; BC, bladder capacity; MV, micturition volume; RV, residual volume; IP, intermicturition pressure; MIOP (mean intermicturition oscillatory pressure; i.e., mean pressure over entire intermicturition interval minus basal pressure on same animal; see MATERIALS AND METHODS.). a100 μg hSlo/hpcDNA in 1 ml PBS. ‡Significantly different from sham operated, P < 0.05; §significantly different from obstructed + saline injected, P < 0.05, 1-way ANOVA with Newman Keuls post hoc pairwise comparisons.

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hSlo Is Expressed in Injected Rats in Which Bladder Overactivity Is Eliminated and MIOP Is Significantly Decreased

RT-PCR with vector- and hSlo-specific primers revealed expression of hSlo only in rats injected with hSlo/pcDNA3 (Fig. 6). Because the bands were subsequently sequence verified (i.e., the full-length fragment containing both hSlo- and vector-specific regions; see MATERIALS AND METHODS and legend to Fig. 6), it is clear that only hSlo was detected. These molecular data clearly implicate hSlo expression in the amelioration of bladder overactivity in the obstructed animals injected with naked hSlo/pcDNA. As shown in Fig. 7, rSlo is also clearly expressed in rat detrusor myocytes.

DISCUSSION

In an aging population with an ever-increasing life span, urinary incontinence will undoubtedly be a growing medical problem. Despite recent progress, all currently available therapies for bladder overactivity (i.e., urge incontinence) have limited efficacy and/or untoward side effects. Certainly, then, there is considerable opportunity for improvements in the treatment of human bladder dysfunction. Because the physiology of the rat bladder parallels many aspects of the human bladder (10, 22), we have begun studies in the rat model to identify physiologically relevant molecular targets that might lead to the development of bladder-specific treatment options. Moreover, it is clear that the pathophysiology of partial urinary outlet obstruction in the rat model recapitulates many relevant aspects of the corresponding lower urinary tract symptoms observed in humans (39, 42, 44). The noted physiological and pathophysiological similarities make it reasonable to assume that studies on the rat bladder will provide insight into at least some aspects of human bladder physiology and dysfunction.

Rat Model of Bladder Hyperactivity

As reported by others, after 6 wk of partial urethral constriction, the rat bladder became both grossly en-
larged (i.e., hypertrophic; see Table 1) and overactive (see Figs. 3–5 and Table 1). The physiological correlates of this overactivity in vivo are clearly illustrated in the representative cystometric recordings shown in Figs. 3–5. As shown, dramatic and reproducible differences in the characteristics of the micturition reflex are apparent in the obstructed animals compared with their normal counterparts. The contractile responses observed between micturitions are the presumptive correlates of the overactivity thought to be associated with urinary urgency and urge incontinence. In addition, the bladder weight in the obstructed animals was approximately three- to fourfold greater than the corresponding weight in the age-matched sham-operated control rats. Taken together, these observations indicate that this experimental animal model may provide a relevant physiological recapitulation of at least some aspects of urge incontinence, i.e., the corresponding human condition.

### Effects of Gene Therapy with a Single Bladder Injection of hSlo/pcDNA on Bladder Overactivity and Contractility

As illustrated in Figs. 4 and 5, a single bladder instillation of hSlo/pcDNA (100 μg in 1 ml PBS) resulted in expression of hSlo mRNA and, furthermore, was associated with the nearly complete elimination of bladder overactivity. Specifically, in contrast to the PBS-injected, obstructed rats, the hSlo-injected, obstructed rats exhibited little or no spontaneous bladder activity between micturitions. The ability of hSlo expression to ameliorate bladder overactivity was further reflected by the fact that statistical analysis of a quan-

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**Fig. 6. Detection of the hSlo transcript.**

A: recombinant hSlo from the rat bladder injected with 100 μg hSlo/pcDNA was examined by RT-PCR, followed by transblotting the RT-PCR products onto nylon membrane and ethidium bromide detection (see MATERIALS AND METHODS). Only a single band was detected, and only in hSlo/pcDNA3-injected animals. The cDNA sequence of this band was also confirmed by DNA sequencing. Each lane represents samples taken from distinct rat bladders (i.e., n = 2). B: another gel, this time with 4 lanes, again with each lane representing a sample from a distinct rat (i.e., n = 4). In this case, an incomplete RT-PCR fragment was also detected. Nonetheless, subsequent sequencing of the 229-bp bands in the Albert Einstein College of Medicine Core Sequencing Facility revealed that they were indeed the expected vector-specific hSlo DNA sequences. C: DNA sequence of the entire expected 229-bp fragment (see MATERIALS AND METHODS for more details). The T7 promoter region of the pcDNA3 vector and the first 18 bp (i.e., the first 6 amino acids) of hSlo were used as the primer pair for RT-PCR. The DNA sequences in this region are unique to the pcDNA3/hSlo and, therefore, are not found in the rat maxi-K channel. As such, the presence of this transcript is a clear indication of the presence of hSlo in rat bladder after pcDNA3/hSlo injection.
A quantitative index of intermicturition activity (i.e., MIOP) revealed that the hSlo-injected, obstructed rats had a mean MIOP value that was significantly lower than that observed for the PBS-injected, obstructed rats, but not different from the mean value of sham-operated control rats (Table 1). As such, both visual inspection of the cystometrograms and statistical analysis of a quantitative measure of bladder overactivity (i.e., MIOP) document the efficacy of hSlo gene therapy. Moreover, hSlo gene therapy achieves this effect without altering many other relevant measures of bladder function.

In addition, as shown in Table 1, the residual volume was significantly higher in the hSlo/pcDNA3-injected, obstructed animals, than in both the PBS-injected, obstructed animals and the control animals. This latter observation is also consistent with an increased detrusor myocyte expression of hSlo, such that there was diminished maximal bladder contractility. The clinical correlates of this observation, if any, are uncertain and, therefore, remain to be determined.

What Is the Cellular Locus of Action of hSlo?

Certainly, based on these preliminary data, we cannot unequivocally discern the cellular locus of action of hSlo gene therapy in these studies. In fact, the extent of LacZ staining we observed in response to a single intravesicle administration of naked DNA may seem somewhat surprising in light of the tight barrier provided by the urothelium. In this regard, it is conceivable that even the apparently modest fluid injection protocol we employed (1 ml/30 s) might be viewed as somewhat disruptive to the urothelium, because in animals of this sex, age, and weight the bladder would experience urine production of approximately 1/10 to 1/50 of that amount (i.e., a 1- to 3-ml bladder capacity, with micturitions every 20–30 min).

However, despite these considerations, the LacZ staining shown in Figs. 1 and 2 is consistent with at least three cogent possibilities. First, it is conceivable that hSlo expression could act similarly to the situation in the rat model of erection in vivo, where hSlo expression presumably alters the cellular excitability of myocytes (i.e., a myogenic effect). A second possibility is that hSlo expression in the urothelial cells lining the bladder wall is associated with increased release of an epithelium-derived relaxing factor, thus buffering bladder hyperactivity. This seems a reasonable suggestion in light of the important putative role of the urothelium in modulating bladder tone (36, 43) and given the generally important role played by maxi-K channels in the synthesis/release of endothelium- and epithelium-derived relaxing factors (20, 33, 38). Yet a third possibility is that hSlo expression alters the excitability of neuronal afferents (i.e., a neurogenic effect), thus diminishing the putative increase in the signal for the myocytes to contract (11, 19). Although we cannot yet distinguish among these three possibilities, we do favor the first two interpretations, although we cannot rule out the latter, as the level of resolution used in these histological investigations did not permit adequate visualization of neural tissue.

Why Does Gene Therapy with hSlo Ameliorate Bladder Overactivity?

The myocyte hypothesis. A recent publication has documented that expression of hSlo in a fraction of the specialized vascular smooth muscle cells of the rat corpus cavernosum (i.e., corporal myocytes) was sufficient to restore the age-related decline in the nerve-stimulated intracavernous pressure response typically observed in older rats (15). The mechanism of action was hypothesized to be related to the enhanced hyperpolarizing ability of the corporal smooth muscle cell network provided by hSlo in response to cellular activation after the release of neurotransmitters (e.g., nitric oxide). The fact that relatively low-level hSlo transfection rates were able to produce dramatic changes in erectile capacity (i.e., intracavernous pressure) was presumed to be related to the presence and physiological relevance of the intercellular pathway provided by connexin43 (Cx43)-derived gap junction channels (13, 14). As such, not every corporal smooth muscle cell needed to be genetically modified to achieve a global effect on tissue function. In this scenario, a certain degree of inefficiency in the transfection process was permissible.

Fig. 7. Expression of endogenous transcript (rSlo) mRNA in rat bladder was also detected via Northern blot analysis.
With respect to the application of hSlo myocyte gene therapy to bladder function, one might not, at first glance, anticipate a similar degree of physiological success. One obvious reason for skepticism is related to the apparent absence of the morphological and structural correlates of gap junctions in human (18, 21) and rat bladder (28, 29). In this regard, a recent report has shown that mRNA for the gap junction protein Cx43 can be detected in the normal rat bladder using RT-PCR techniques (16). Moreover, it is clear that the absence of morphologically detectable gap junctional plaques in rat bladder does not preclude a physiologically relevant contribution of the intercellular pathway to bladder function (9, 16, 24). Finally, as substantially increased Cx43 mRNA levels have recently been reported in the 6-wk obstructed rat bladder (at least 3- to 5-fold; see Ref. 16), one might anticipate that this increased intercellular communication would complement, and perhaps even further augment, the efficacy of hSlo gene therapy in the rat bladder.

In addition, it is becoming increasingly evident that strategic clusters of KCa channels in close proximity to the ryanodine-sensitive calcium stores of the underlying sarcoplasmic reticulum provide an important mechanism for the local modulation of calcium sparks and membrane potential in diverse smooth muscle, including urinary bladder (35). If a similar physiological scenario exists in detrusor myocytes of the rat bladder, and, furthermore, if this regulatory system is altered in the obstructed bladder, then perhaps the K\(^+\) currents carried by hSlo act in a manner that alters intracellular calcium mobilization and/or spread in a sufficient fraction of affected cells to counteract the pathophysiological changes that result in bladder overactivity (e.g., altered electrical properties in myocytes/neurons resulting from changes in ion channel expression, regulation, or function; Refs. 8, 17, 26, 41, 45, 47, 51, and 54). That is, perhaps expression of hSlo is able to short-circuit the intracellular calcium changes that provide the trigger for spontaneous bladder contractions.

The epithelial cell hypothesis. As alluded to above, the maxi-K channel is thought to be important to both epithelium- and epithelium-dependent relaxation responses of underlying smooth muscle cells. In both cell types the central hypothesis is that the maxi-K channel plays a role in the synthesis and/or release of a smooth muscle relaxing factor(s), albeit by a relatively ill-defined mechanism at present. With respect to the regulation of vasomotor tone, the endothelial maxi-K channel has been shown to be critical to nitric oxide-mediated, endothelium-dependent relaxation responses (20, 33, 38). Similarly, the maxi-K channel is thought to be involved in a nitric oxide-independent, but still epithelium-dependent, relaxation of human bronchial smooth muscle (49). Interestingly, both nitric oxide-dependent and -independent (nonnitrergic) mechanisms may exist in the bladder (36, 43). Regardless of the identity of the substance so released, the main implication is that the increased expression of hSlo might result in an increased sensitivity of these cells to release this substance during, for example, the stretching associated with bladder filling. In fact, quiescence of the bladder musculature during filling is indeed thought to be one of the explicit purposes of epithelium-derived relaxing substances. In this scenario, increasing either the sensitivity and/or amount of the relaxing substance released by urothelial (i.e., epithelial) cells would functionally antagonize the underlying bladder hyperactivity.

The neural hypothesis. Because both neurogenic (2, 11, 19) and myogenic (5, 26, 41, 51) mechanisms may play a role in the etiology of bladder hyperactivity, it is also quite clear that neural mechanisms, i.e., increased afferent nerve activity, may be relevant. If so, then neuronal hSlo expression might be expected to affect neural activity either directly or indirectly, the former by virtue of the effects of increased release of an epithelium-derived relaxing factor on the underlying neural afferents (see above) and the latter by directly increasing the hyperpolarizing ability of the neuralafferent as described above for the detrusor myocyte. Thus, while we detected no evidence for a direct effect of gene therapy on neural tissue, we cannot unequivocally rule out the possibility.

Taken together, these studies further document the important role of hSlo-mediated hyperpolarizing currents to the modulation of bladder function at a variety of possible levels and, furthermore, indicate that gene therapy may be an effective, perhaps even ideal, therapeutic modality for the treatment of bladder hyperactivity (and perhaps the amelioration of ensuing urinary urgency and incontinence), as previously suggested for erectile dysfunction (15).

Perspectives

The mechanistic basis for bladder hyperactivity may involve neural or myocyte mechanisms or, perhaps most likely, both. The optimal treatment of bladder hyperactivity then would presumably be dependent on more precise knowledge of the etiology. However, current understanding of bladder function, in conjunction with the studies described in this report, indicates that gene therapy with hSlo may coincidentally provide an ideal treatment option for bladder hyperactivity, largely independent of cause. In fact, one might even conclude that the hyperactive rat bladder is perhaps an ideal target for low-efficiency gene transfer. More specifically, depending on the precise distribution and/or compartmentalization of the hSlo channels, increased expression of the calcium-sensitive (hSlo) K channel could well serve as an ideal physiological antagonist of spontaneous detrusor contractions at several distinct levels: 1) by increasing the sensitivity of the detrusor myocytes to neuronally released or stretch-induced relaxing substances (both epithelium derived and myocyte derived; Ref. 23) and/or permitting the detrusor myocytes to short-circuit pacemaker cell-induced calcium signals and/or calcium waves among the detrusor myocytes, 2) by increasing the amount of epithelium-derived relaxing substance(s) released by the urothe-
lium in response to stretch-induced filling, and 3) by diminishing the putative increased activity of neuronal afferents. In fact, regardless of the cellular locus and precise mechanism of disease, hSlo expression clearly ameliorates bladder hyperactivity in this rat model. Further work remains to be done to unequivocally discern the mechanism and document the verity of these exciting possibilities.

REFERENCES

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