Neurourology and Urodynamics

Alterations in Bladder Function Associated With Urothelial Defects in Uroplakin II and IIIa Knockout Mice

Tamer Aboushwareb,[1] Ge Zhou,[2] Fang-Ming Deng,[2] Chanda Turner,[1] Karl-Erik Andersson,[1,3] Moses Tar,[4] Weixin Zhao,[1] Arnold Melman,[4] Ralph D'Agostino Jr.,[5] Tung-Tien Sun,[3]** and George J. Christ[1,3,6]*

[1]*Wake Forest Institute for Regenerative Medicine, Wake Forest University School of Medicine, Winston-Salem, North Carolina*

[2]*Departments of Cell Biology, Dermatology, Urology and Pharmacology, New York University School of Medicine, New York, New York*

[3]*Department of Urology, Wake Forest University School of Medicine, Winston-Salem, North Carolina*

[4]*Albert Einstein College of Medicine, Bronx, New York*

[5]*Public Health Sciences, Wake Forest University School of Medicine, Winston-Salem, North Carolina*

[6]*Department of Physiology & Pharmacology, Wake Forest University School of Medicine, Winston-Salem, North Carolina*

Aims: The effects of deleting genes encoding uroplakins II (UPII) and III (UPIIIa) on mouse bladder physiology/dysfunction were studied in male and female wild type and knockout (KO) mice. **Methods:** UPII, UPIIIa, and WT mice were catheterized using previously described techniques. Continuous cystometry was conducted in conscious, freely moving animals. Bladder strips were harvested after animal sacrifice and pharmacological studies and EFS were conducted in an organ chamber. Histological studies were also carried on with H&E staining to identify differences among the three mouse types. **Results:** These studies have revealed numerous alterations, some of which were apparently gender-specific. Nonvoiding contractions were common in both UPII and UPIIIa KO mice, although more severe in the former. In particular, the increased bladder capacity, micturition pressure and demonstrable nonvoiding contractions observed in the male UPII KO's, were reminiscent of an obstruction-like syndrome accompanied by evidence of emerging bladder decompensation, as reflected by an increased residual volume. Pharmacological studies revealed a modest, gender-specific reduction in sensitivity of isolated detrusor strips from UPII KO female mice to carbachol-induced contractions. A similar reduction was observed in UPIIIa KO female mice. Histological investigation showed urothelial hyperplasia in both UPII KO and UPIIIa KO mice, although again, apparently more severe in the former. **Conclusions:** These results confirm and extend previous work to indicate that urothelial defects due to uroplakin deficiency are associated with significant alterations in bladder function and further highlight the importance of the urothelium to bladder physiology/dysfunction. *Neurourol. Urodynam.* © 2009 Wiley-Liss, Inc.

Key words: BOO; LUTS; urodynamics

INTRODUCTION

During the past decade knockout (KO) mice have become increasingly useful animal models for studying molecular mechanisms underlying various forms of lower urinary tract (LUT) disease.[1] Some recent examples of particular relevance to bladder physiology/dysfunction include the development of KO mice for nitric oxide synthase, as well as purinergic and prostaglandin receptor subtypes, K channels and caveolins.[2–10] These models have clearly demonstrated that molecular alterations to the nerves and smooth muscle cells of the bladder wall alter bladder function. More recent evidence documenting the importance of the urothelium to modulation of bladder physiology/dysfunction has also generated interest in urothelium-specific KO mice. One such model is the uroplakin KO mouse, the subject of this investigation.

In this regard, uroplakins are integral components of the plaques lining the urothelium and are critical to normal urothelial barrier function, and thus, normal genitourinary function.[11–13] In fact, the apical surface of terminally differentiated mammalian urothelial umbrella cells is covered by numerous plaques consisting of two-dimensional crystals of hexagonally packed 16 nm uroplakin particles.[14,15] There are four major uroplakins (Ia, Ib, II, and IIIa), and one minor form (IIIb).[16,17] They initially assemble as heterodimers, in pairs of Ia/II and Ib/III, only then they can exit from the endoplasmic reticulum and reach the cell surface.[1,11,13,18–20]

In fact, recent studies in uroplakin IIIa KO mice have demonstrated the presence of an abnormal urothelial umbrella cell layer characterized by irregular, small plaques.[11] As both heterodimers (Ia/II and Ib/IIIa) are required for normal plaque formation, it has been postulated that uroplakin IIIb replaces the necessary function of the UPIIIa to form Ib/IIIb heterodimer, allowing for the small crystal plaques to develop.[13] Knockout of uroplakin II, which does not have a known isoform, leads to the complete absence of plaque

Conflicts of interest: none

Tamer Aboushwareb and Ge Zhou contributed equally to this work.

Lori Birder led the review process.

*Correspondence to: George J. Christ, Ph.D., Wake Forest Institute for Regenerative Medicine, Wake Forest University School of Medicine, Medical Center Boulevard, Winston Salem, NC 27157. E-mail: gchrist@wfubmc.edu

**Correspondence to: Tung-Tien Sun, Ph.D., Department of Cell Biology, NYU Medical School, 550 First Av, New York, NY 10016. E-mail: sunt01@med.nyu.edu

Received 12 August 2008; Accepted 10 November 2008

Published online in Wiley InterScience (www.interscience.wiley.com)

DOI 10.1002/nau.20688

formation, supporting the idea that the two uroplakin heterodimers are both critical for plaque formation.[13]

The major importance of uroplakins to lower urinary tract function is apparent from previous work documenting that uroplakin defects lead to compromised urothelial permeability barrier, vesico-ureteral reflux, and abnormal voiding patterns.[11–13] However, the direct impact of uroplakin deficiency/defects on bladder phenotype and function, as evaluated by urodynamic studies in vivo, has not yet been characterized. In this regard, the main aim of the present study was to determine the effects of gene deletion of uroplakin II (UPII KO) and IIIa (UPIIIa KO) on mouse bladder function as determined by continuous cystometry in conscious animals.

MATERIALS AND METHODS

The UPII KO and UPIIIa KO mice used at 2–3 months old were produced as previously described.[11,13] They were housed in a barrier facility at the Institute for Animal Studies, Albert Einstein College of Medicine with a 12-hr light/dark cycle, and free ad libitum access to Picolab 20 chow (PMI Nutrition International, Gray Summit, Missouri) and water. All studies were performed under the approval of the IACUC at Wake Forest university school of medicine.

Animal Surgery

Surgical procedures in the mice were performed as previously described.[10,21] Briefly, control and KO mice were anesthetized via intraperitoneal injections of xylazine (7–14 mg/kg) and ketamine (37.5–75 mg/kg) formulated for animal use. The ventral abdominal wall, perineum and upper back were shaved with an electrical shaver and cleansed with povidone–iodine. A low midline abdominal incision was made and the bladder was identified. A small incision was made in the bladder dome and a polyethylene (PE-10, Clay Adams, Parsippany, New Jersey) catheter with a cuff was inserted. A 6-0 silk suture was placed around the catheter to anchor it and close the bladder incision. Saline was injected through the catheter to ensure no bladder leakage. The catheter was then tunneled through the subcutaneous space and exited through an orifice created in the back of the animal and secured with a suture. The abdominal incision was then closed using a 5-0 silk suture and the free end of the catheter was thermally sealed.

Cystometric Analysis

Analysis of cystometric data was performed in a manner similar to that previously described.[10,21–23] Three days after bladder catheter implantation conscious mice were placed in a mouse metabolic cage and the bladder catheter was connected to a two-way valve connected to a pressure transducer and infusion pump. The pressure transducer was connected via an ETH 400 (CB Sciences, Dover, New Hampshire) transducer amplifier to a MacLab/8e (Analog Digital Instruments, Castle Hill, New South Wales, Australia) data acquisition board. The pressure transducers and analog-to-digital board were calibrated in cmH_2O before each experiment. Room temperature saline was infused into each bladder at a rate of 1.5 ml/hr. Micturition volumes were measured with a silicone coated collecting funnel that directed urine into a collecting tube connected to a force displacement transducer. Intravesical pressure and micturition volumes were continuously recorded. Analysis began after the voiding pattern of the mice stabilized. Only mice with baseline pressures less than 20 cmH_2O and 30–60 min of reproducible micturition cycles were analyzed.

Table II lists all cystometric parameters measured. Bladder function was evaluated by the following urodynamic parameters: (1) bladder capacity (BC), the volume of infused saline at micturition; (2) basal pressure (BP), the lowest bladder pressure recorded during cystometry; (3) threshold pressure (TP), the bladder pressure immediately before micturition; (4) micturition pressure (MP), the peak bladder pressure during micturition; (5) micturition volume (MV), the volume of urine discharged during micturition; (6) residual volume,[24] the volume of infused saline minus the micturition volume; (7) intermicturition pressure (IMP), the mean pressure between micturitions; (8) spontaneous activity (SA), an approximate index of spontaneous bladder contractions between micturitions (i.e., detrusor overactivity); SA was calculated by subtracting the BP from the IMP. Note that this parameter is identical to that previously described as mean intermicturition oscillatory pressure ($MIOP = IP - BP$);[21] (9) bladder compliance (B_{Com}), the change in pressure between voiding contractions, and (10) micturition frequency.

Pharmacological Studies

Bladders from a subset of WT, UPII KO, and UPIIIa KO mice were harvested and cut into equal size strips along the longitudinal axis (3–4 strips/mouse). The strips were immediately mounted in a 7 ml organ bath myograph system (Danish Myo Technology, Aarhus, Denmark) containing Krebs-Henseleit buffer (110 mM NaCl, 4.8 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, 11 mM glucose and 11 mM dextrose). The organ chambers were maintained at a mean ± SEM of 37 ± 0.05°C with continuous 95% O_2 and 5% CO_2. The bladder strips were subjected to a resting tension of 300 mg and isometric tension was recorded using a transducer coupled to a MacLab data acquisition system. The bladder strips were allowed to equilibrate for 60 min and washed with fresh buffer every 15 min. Contractions were recorded as steady-state changes in tension from baseline with increasing concentrations of carbachol at ½ log increments (ranging from 2×10^{-8} to 5×10^{-5} M). Carbachol concentration-response curves were computer fitted to the four parameter logistic equation using Prism 4.0 software (GraphPad Software, Inc., San Diego, CA).

Histological Evaluation

Urinary bladders were dissected from a subset of experimental animals. To prepare paraffin embedded sections, the bladders were inflated to a uniform volume with 0.1 ml of 10% neutral buffered formalin for fixation. Coronal sections of the bladder were obtained from the base, mid-section and neck of the bladder. Sections were cut at 4 μm and were stained routinely with hematoxylin and eosin.

Statistical Analysis

Statistical analysis was performed using Systat for Windows (Systat v., Systat Software, San Jose, CA). Unless otherwise stated, all data are presented as the mean ± standard error of the mean (SEM). Individual outcome measures were examined for normality and when needed a natural log transformation was used. Two-way ANOVA models were fit with gender and group (i.e., WT, UPII or UPIIIa KO mice) included as factors. The group by gender interaction

was examined in this model, and if found to be significant, analyses were performed with the inclusion of the interaction term. In all cases where ANOVA revealed significant differences, appropriate post hoc statistics were utilized, with $P < 0.05$ considered significant.

RESULTS

Demographics, Body, and Bladder Weights

The distributions of mice in the various experimental animal groups are provided in Table I, along with the mean data on bladder and body weights and bladder weight/body weight ratio. Two-way ANOVA revealed the following: (1) in all groups males had larger body weights than females; (2) both male and female UPII KO mice had lower body weights than their WT counterparts; (3) the UPII KO and UPIIIa KO males had larger bladder weights than their female counterparts and the male WT mice; and (4) the UP KO mice in general had higher bladder weight to body weight ratios than their WT counterparts. This phenomenon may be attributed to the apparent enlargement of the bladder of these animals, even though light microscopy did not reveal significant differences in the overall histology in bladder cross-sections.

Urodynamic Evaluation of Bladder Function in WT and KO Mice

Two-way ANOVA following digital analysis of cystometric records revealed a host of statistically significant differences in a variety of cystometric parameters, both between genders and among various experimental mouse groups. All cystometric data (mean ± SEM) and associated statistical inferences are summarized in Table II, and representative tracings are shown for each group in Figure 1. For clarity and simplicity, only the major physiological findings are reviewed below.

As illustrated, the WT male and female mice each displayed quite regular voiding intervals with clear micturitions and only minor, if any, pressure fluctuations between micturitions (Fig. 1). In stark contrast, cystometric records from both UPII and UPIIIa KO mice (both males and females; Fig. 1) revealed demonstrable nonvoiding contractions (i.e., detrusor over activity (DO)). Consistent with these observations, two-way ANOVA revealed that the mean spontaneous activity value for all male and female UP KO mice was significantly greater than that observed in the WT mice. In addition, the UPII KO mice displayed significant elevations in spontaneous activity, intermicturition pressure and micturition pressure relative to both the WT and UPIIIa KO mice. The UPIIIa KO mice, in turn, had lower basal pressure and threshold pressure values than either the UPII or WT mice. In short, the observed DO was more pronounced (both qualitatively and quantitatively) in the UPII KO mice than the UPIIIa KO mice (Fig. 1). Two-way ANOVA also showed that the male UPII KO mice had significant elevations in bladder capacity and residual volume relative to other groups. More specifically, on average, the residual volume of the UPII KO males was approximately 37% of the total bladder capacity.

Pharmacological Studies of Muscarinic-Mediated Detrusor Contractility in WT and UP KO Mice

To examine potential myogenic contributions to the observed UP KO-related urodynamic changes, concentration-response curves for steady-state carbachol-induced contractions were performed on urothelium-denuded detrusor strips derived from bladders on a subset of the total study population. All curves were computer fit to the logistic equation to derive E_{max}, pEC_{50}, and slope factor values (Table III). Two-way ANOVA of mean logistic parameter estimates revealed modest, albeit statistically significant, decreases in sensitivity (pEC_{50}) to carbachol-induced contractions in female UPII KO mice relative to their male counterparts as well as the female WT mice. A similar reduction in the pEC_{50} value was observed for the female UPIIIa KO mice, also relative to the WT females.

Bladder Tissue Architecture in WT and UP KO Mice

Histological studies (H&E) were also conducted on bladder tissue from a subset of the animal population in order to gain further mechanistic insight into the potential role of uroplakin deficiency on bladder function. Representative examples of the histological findings from UPII KO male (n = 2) and female (n = 4) and UPIIIa KO male (n = 3) and female (n = 5) bladders are displayed in Figures 2 and 3, with histologic findings from WT male and female mice also shown for comparison. As illustrated, when compared to WT mice (Figs. 2A,B and 3A,B), UPII KO (Figs. 2C,D and 3C,D), and UPIIIa (Figs. 2E,F and 3E,F) KO mice displayed a markedly hyperplastic urothelium that also appeared to have an atypical superficial cell layer. While these findings were conspicuous in mice of both genders, they appeared somewhat more pronounced in bladders from the female KO mice (Fig. 2) than in bladders from males (Fig. 3). In addition, the urothelial thickening in UP KO bladders was not uniform, but rather, varied regionally. There were no obvious or striking differences in the bladder wall of mice from any group. These observations are consistent with previous work documenting similar findings in other portions of the lower urinary tract.[11,13,16]

TABLE I. Summary of Bladder and Body Weights in WT and UP KO Mice

Group	Gender	Bladder weight (g)	Body weight (g)	Bladder weight/body weight
WT	Male (n = 11)	$0.748 \pm 0.03^{e,f}$	43.5 ± 1.0^{a}	$0.017 \pm 0.001^{b,e,f}$
	Female (n = 10)	$0.734 \pm 0.04^{e,f}$	36.5 ± 0.9	$0.02 \pm 0.001^{b,e}$
UPII KO	Male (n = 9)	$0.898 \pm 0.04^{a,b,c,d}$	36 ± 0.7^{b}	$0.025 \pm 0.001^{a,c,d}$
	Female (n = 10)	0.703 ± 0.0392	26.5 ± 0.6	$0.027 \pm 0.001^{a,c,d,f}$
UPIIIa KO	Male (n = 10)	$0.923 \pm 0.07^{a,b,c,d}$	44 ± 1.8^{c}	$0.022 \pm 0.001^{b,d}$
	Female (n = 9)	$0.629 \pm 0.03^{e,f}$	34.9 ± 1.1	$0.018 \pm 0.001^{b,e}$

Data are expressed as mean ± SEM. Statistical analysis of differences between groups was performed using two-way ANOVA. Values of $P \leq 0.05$ were taken as statistically significant. [a]Significantly different from WT female. [b]Significantly different from UPII KO female. [c]Significantly different from UPIII KO female. [d]Significantly different from WT male. [e]Significantly different from UPII KO male. [f]Significantly different from UPIII KO male. Red Fill: Group significantly different from other groups.

TABLE II. Comparison of Mean Cystometric Parameters in WT and UP KO Mice

Group	Sex	BC (ml)	MV (ml)	RV (ml)	BP (cmH_2O)	TP (cmH_2O)	MP (cmH_2O)	IMP (cmH_2O)	SA (cmH_2O)	B_{com} (ml/cmH_2O)	MF (ml/hr)
WT	Male (n = 11)	0.29 ± 0.05^{a}	0.23 ± 0.05^{a}	0.07 ± 0.01^{e}	6.23 ± 1.17	15.30 ± 2.11	38.18 ± 2.78	10.10 ± 1.45	3.37 ± 0.52	$0.05 \pm 0.09^{a,b}$	$8.5 \pm 1.5^{a,e}$
	Female (n = 10)	$0.17 \pm 0.03^{c,f}$	$0.11 \pm 0.02^{a,d,e,f}$	0.06 ± 0.02^{e}	8.82 ± 1.68	18.31 ± 1.61	35.03 ± 2.88	13.11 ± 2.03	4.29 ± 0.63	$0.02 \pm 0.003^{c,e,f}$	$13.9 \pm 3.1^{c,d,e,f}$
UPII KO	Male (n = 9)	$0.54 \pm 0.15^{*}$	$0.34 \pm 0.07^{a,b}$	$0.2 \pm 0.08^{a,b,d,f}$	7.47 ± 2.09^{b}	$17.03 \pm 2.27^{b,e}$	41.61 ± 3.82	14.21 ± 3.31^{b}	6.74 ± 1.40^{b}	$0.05 \pm 0.01^{a,b}$	$5.4 \pm 1.1^{a,b,d}$
	Female (n = 10)	0.18 ± 0.02	0.11 ± 0.01^{e}	$0.04^{e} \pm 0.01$	12.38 ± 1.92^{f}	23.55 ± 2.48^{f}	46.20 ± 4.82	23.23 ± 2.78	10.86 ± 1.16	$0.02 \pm 0.002^{c,e,f}$	10.6 ± 0.9^{e}
UPIIIa KO	Male (n = 10)	0.25 ± 0.02^{a}	0.19 ± 0.02^{a}	$0.06^{e} \pm 0.01$	3.83 ± 1.56^{c}	11.29 ± 1.25^{c}	35.65 ± 1.9	8.14 ± 2.49	4.31 ± 0.97	$0.04 \pm 0.003^{a,b}$	7.2 ± 0.9^{a}
	Female (n = 9)	0.25 ± 0.02^{a}	0.18 ± 0.02^{a}	0.06 ± 0.01	5.31 ± 0.1	13.53 ± 0.81	35.38 ± 1.77	12.78 ± 2.29	7.47 ± 1.40	$0.03 \pm 0.002^{a,b}$	7.0 ± 0.4^{a}

BC, bladder capacity, ml; MV, micturition volume, ml; RV, residual volume, ml; BP, basal pressure cmH_2O; TP, threshold pressure, cmH_2O; MP, micturition pressure, cmH_2O; IMP, intermicturition pressure, cmH_2O; SA, spontaneous activity, IMP-BP; B_{com}, bladder compliance, ml/cmH_2O; MF, micturition frequency. Data are expressed as mean ± SEM. Statistical analysis of differences between groups performed using two-way ANOVA. Values of $P \leq 0.05$ are taken as statistically significant. Where [a]Significantly different from WT female. [b]Significantly different from UPII KO female. [c]Significantly different from UPII KO female. [d]Significantly different from WT male. [e]Significantly different from UPII KO male. [f]Significantly different from UPIII KO female. Red fill: group significantly different from other groups. Yellow fill: group significantly different from WT. Light blue fill highlights the volume differences observed in the UPII KO male.
*Significantly different from ALL other BC values.

DISCUSSION

Normal bladder function is dependent on an orchestration of neural, myogenic and urothelial mechanisms. Perturbations of any of these mechanisms can lead to disruption of bladder function. Not surprisingly then, bladder dysfunction, including DO, is known to be a multifactorial disease process. A major goal of this investigation, therefore, was to utilize the UP KO mouse model to begin to examine the potential contributions of the urothelium to normal bladder physiology/dysfunction.

The major implication of the present data is that they clearly document that uroplakin deficiency (due to the ablation of the UPII and UPIIIa genes), and in particular the absence of UPII, is associated with significant changes in bladder function in the mouse model as determined by urodynamic evaluation. The alterations in cystometric parameters in both UPII KO and UPIIIa KO mice (Fig. 1; Table II) were associated with rather unique structural changes (Figs. 2 and 3) [11,12] as well as modest pharmacological alterations (Table III). Some of the observed changes were apparently gender-specific. Since the number of differences detected among the three groups and between the two genders was quite large (see Tables I–III for details), we will focus our attention on only the most physiologically relevant observations.

With respect to UPII, prior work documents that UPII KO mice have no plaque formation, with a significantly delayed excretion of Omnipaque, indicative of ureteral obstruction.[13] In these earlier studies, serial sectioning of ureters of UPII KO mice revealed areas with epithelial polyps or complete epithelial occlusion, resulting in an anatomic obstruction. Such observations suggested that retrograde flow of urine from the bladder into the ureters (i.e., vesicoureteral reflux) as well as structural and/or functional obstruction of the ureters may account for the observed hydronephrosis in UPII KO animals.[11,13] Consistent with these earlier findings, the present study revealed that the bladder urothelium of the UPII KO (and UPIIIa, see below) mice exhibited substantial hyperplasia, and furthermore, apparently showed an atypical superficial cell layer (Figs. 2 and 3).[11,13] These urothelial changes are known to contribute to defective urothelial permeability,[12] which may in turn lead to DO.

Consistent with this interpretation is the fact that the UPII KO mice, which have more severe histological and ultrastructual defects than the UPIIIa KO mice,[11,13] displayed a higher incidence of nonvoiding contractions (Fig. 1). Moreover, in line with these urodynamic observations, analysis of the digital records revealed that the UPII KO mice had significant elevations in both intermicturition pressure and spontaneous activity (spontaneous activity (SA) is a quantitative measure of DO, see Materials and Methods Section) relative to both the WT and UPIIIa KO mice (Table II). Furthermore, in the male UPII KO mice, the elevated pressure changes (micturition pressure, intermicturition pressure and spontaneous activity) were further accompanied by an increased bladder capacity and residual volume (Table II). Although further work is clearly necessary, taken together, these initial data indicate the presence of impending bladder decompensation in the male UPII KO mouse, possibly due to the existence of an obstruction-like syndrome related to the UPII KO-mediated urothelial perturbations. Interestingly, the bladders of the female UPII KO's, despite even larger apparent pressure alterations, were still able to empty normally, at least as reflected by the fact that residual volume remained unaltered.

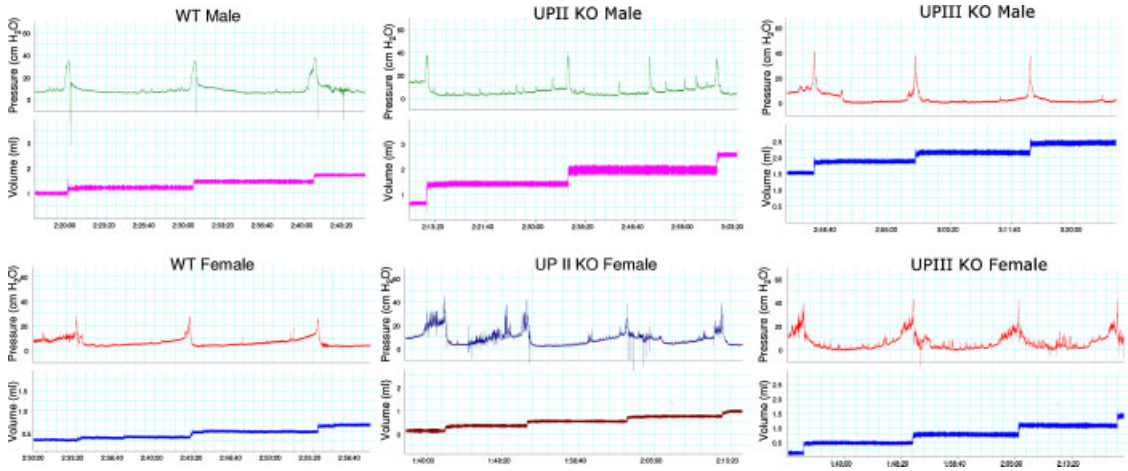

Fig. 1. Representative cystometric recording from a male and female WT, UPII KO and UPIII KO mice. Note the regular micturition pattern and the absence of significant pressure fluctuations between micturitions (i.e., few, if any, nonvoiding contractions) in the WT mice. In contrast, note that the micturition patterns in the UPII and UPIIIa KO mice were quite irregular with demonstrable pressure fluctuations between micturitions (i.e., nonvoiding contractions). These nonvoiding contractions were the presumptive clinical correlates of urgency and as such were not associated with bladder emptying. *Y* axis units are in H:M:S.

Findings similar to those described above for the UPII KO mouse have been reported in UPIIIa KO mice as well.[11,12] In fact, the urothelium of the UPIIIa KO mouse also has an atypical superficial urothelial cell layer in the bladder. A deficit of UPIIIa is associated with a reduced urothelial plaque size, compromised urothelial permeability barrier function, and vesicoureteral reflux resulting in hydronephrosis.[12] Furthermore, as reported herein (Figs. 2 and 3), these earlier studies also documented the presence of urothelial hyperplasia in the bladder. As summarized in Table II and illustrated in Figure 1, the UPIIIa KO mice also displayed a significant increase in nonvoiding contractions compared to their WT counter parts (Fig. 1). Of note, the UPIIIa KO mice had significantly lower values of BP and TP than either the UPII KO or WT mice. As such, it is intriguing to speculate that the increased incidence of nonvoiding contractions observed in the UPIIIa KO mice might be related to the apparently decreased pressures required to initiate micturition (i.e., decreased TP).

Another potential impact of urothelial deficiency is increased excitability of the detrusor myocytes during filling of the bladder. Such secondary alterations, either alone, or in parallel with neurogenic or other local changes (i.e., urothelial) may enhance spontaneous contractile activity,[24] thus promoting the appearance of the nonvoiding contractions characteristic of DO. In order to gain some initial insight into this possibility, we conducted pharmacological studies to examine steady-state carbachol-induced contractions on equivalent sized bladder strips from mice of all groups and both genders. Logistic analysis of the data revealed that muscarinic-mediated contractile responses were largely unaltered, with the exception of relatively modest changes in the pEC_{50} values, such that the pEC_{50} in detrusor strips from female UPII KO mice was lower than that observed in their male counterparts or WT female mice. Again, a similar reduction in the pEC_{50} value was observed for the female UPIIIa KO mice, also relative to the WT females. These data indicate that muscarinic-mediated contractile responses in UP KO mice are either unchanged (males) or less sensitive (females), and thus, it would appear that enhanced contractility to endogenous neurotransmitters does not contribute greatly to the altered bladder function (i.e., increased spontaneous activity, intermicturition pressure and DO) observed in the UP KO mice. Future studies examining the impact of uroplakin deficiency on nerve-stimulated contractile responses of isolated detrusor

TABLE III. Logistic Analysis of Cumulative Steady-State Carbachol-Induced Dose–Response Curves in Wild Type, Uroplakin II, and Uroplakin IIIa Knockout (KO) Mice

Group	Gender	E_{max} (g)	pEC_{50}	Slope
WT	Male (11)	3.3 ± 0.5	5.73 ± 0.08	1.0 ± 0.1
	Female (11)	3.7 ± 0.5	5.67 ± 0.08	1.1 ± 0.1
UPII KO	Male (13)	3.3 ± 0.5	5.63 ± 0.07*	1.0 ± 0.1
	Female (11)	3.9 ± 0.5	5.39 ± 0.08**	1.1 ± 0.1
UPIIa KO	Male (14)	3.6 ± 0.4	5.56 ± 0.07	1.3 ± 0.1
	Female (11)	3.0 ± 0.5	5.40 ± 0.08**	1.3 ± 0.1

The number of mice in each category/group was 4. Statistics were based on the number of strips (shown in parentheses). Data are expressed as mean ± SEM. Statistical analysis of differences between groups was performed using Two-way ANOVA followed by post hoc pairwise comparisons. Values of $P < 0.05$ were taken as statistically significant.
*Significantly different from UPII KO female.
**Significantly different from WT female.

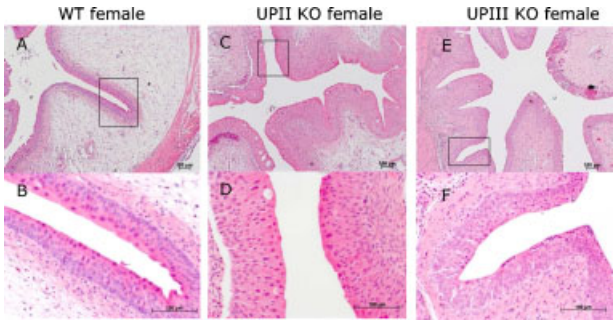

Fig. 2. Representative histological findings in WT, UPII KO, and UPIIIa KO female mice. Panels **A**, **C**, and **E** display representative bladder sections with the corresponding outlined areas shown at higher magnification in Panels **B**, **D**, and **F**. Note the clear differences in urothelial histology between the WT and KO female mice, and also note that the bladder wall was unremarkable for any consistent structural changes in the KO mice.

strips will be needed to further elucidate the mechanistic basis for the observed increases in spontaneous activity and bladder pressures.

In conclusion, these data confirm and extend previous observations to indicate that uroplakin deficiency contributes to abnormal bladder urodynamics. Another interesting observation is that the deletion of the UPII gene, in particular, leads to more severe perturbations of both urothelial histology and bladder urodynamics than that of UPIIIa. This result is consistent with our previous finding indicating that while the knockout of UPIIIa led to a reduction of urothelial plaque formation, it did not completely abolish plaque formation, presumably because UPIIIb, a minor isoform of UPIIIa, can be up-regulated and can form heterodimers with UPIb.[11] On the other hand, ablation of UPII leads to complete elimination of urothelial plaques, probably because UPII has no known isoform.[13] Since our preliminary in vitro pharmacological data revealed relatively modest differences in the steady-state responses of the KO detrusor strips to carbachol, the observed urodynamic changes in the UP KO bladders may reflect a defect in the signaling mechanisms involving superficial bladder structures, that is, urothelium, interstitial cells and/or suburothelial afferent nerves. Finally, it appears that the UPII KO male mouse may provide a valuable model for exploring the potential role of urothelial defects in obstruction-like alterations in bladder function (i.e., a model of LUTS in males).

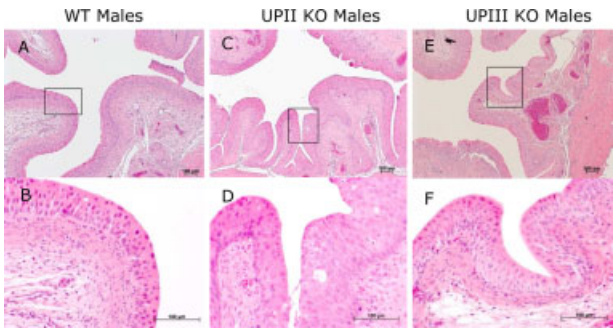

Fig. 3. Representative histological findings in WT, UPII KO, and UPIIIa KO male mice. Panels **A**, **C**, and **E** display representative bladder sections with the corresponding outlined areas shown at higher magnification in Panels **B**, **D**, and **F**. Note the clear differences in urothelial histology between the WT and KO male mice. Once again, note the demonstrable evidence for a UP KO-related urothelial hyperplasia (compare with Fig. 2), as well as the fact that the bladder wall was unremarkable for any consistent structural changes.

REFERENCES

1. Hu TW, Wagner TH, Hawthorne G, et al. Economics of incontinence. In: Abrams P, Cardozo L, Khoury S, Wein A, editors. 3rd International Consultation on Incontinence. Paris: Health Publications Limited; 2005. pp 75–95.
2. Burnett AL, Calvin DC, Chamness SL, et al. Urinary bladder-urethral sphincter dysfunction in mice with targeted disruption of neuronal nitric oxide synthase models idiopathic voiding disorders in humans. Nat Med 1997; 3:571–4.
3. Cockayne DA, Hamilton SG, Zhu QM, et al. Urinary bladder hyporeflexia and reduced pain-related behaviour in P2X3-deficient mice. Nature 2000;407: 1011–5.
4. Cristofaro V, Peters CA, Yalla SV, et al. Smooth muscle caveolae differentially regulate specific agonist induced bladder contractions. Neurourol Urodyn 2007;26:71–80.
5. Gabel BR, Elwell C, van Ijzendoorn SC, et al. Lipid raft-mediated entry is not required for Chlamydia trachomatis infection of cultured epithelial cells. Infect Immun 2004;72:7367–73.
6. Lai HH, Boone TB, Thompson TC, et al. Using caveolin-1 knockout mouse to study impaired detrusor contractility and disrupted muscarinic activity in the aging bladder. Urology 2007;69:407–11.
7. Meredith AL, Thorneloe KS, Werner ME, et al. Overactive bladder and incontinence in the absence of the BK large conductance Ca2+-activated K+ channel. J Biol Chem 2004;279:36746–52.
8. Schroder A, Newgreen D, Andersson KE. Detrusor responses to prostaglandin E2 and bladder outlet obstruction in wild-type and Ep1 receptor knockout mice. J Urol 2004;172:1166–70.
9. Sutherland RS, Kogan BA, Piechota HJ, et al. Vesicourethral function in mice with genetic disruption of neuronal nitric oxide synthase. J Urol 1997; 157:1109–16.
10. Woodman SE, Cheung MW, Tarr M, et al. Urogenital alterations in aged male caveolin-1 knockout mice. J Urol 2004;171:950–7.
11. Hu P, Deng FM, Liang FX, et al. Ablation of uroplakin III gene results in small urothelial plaques, urothelial leakage, and vesicoureteral reflux. J Cell Biol 2000;151:961–72.
12. Hu P, Meyers S, Liang FX, et al. Role of membrane proteins in permeability barrier function: uroplakin ablation elevates urothelial permeability. Am J Physiol Renal Physiol 2002;283:F1200–7.
13. Kong XT, Deng FM, Hu P, et al. Roles of uroplakins in plaque formation, umbrella cell enlargement, and urinary tract diseases. J Cell Biol. 2004; 167: 1195–204.
14. Hicks JB. Introduction to fluid and electrolyte metabolism. Med Rec Ann 1964;57:354 PASSIM.
15. Kachar B, Liang F, Lins U, et al. Three-dimensional analysis of the 16 nm urothelial plaque particle: luminal surface exposure, preferential head-to-head interaction, and hinge formation. J Mol Biol 1999;285:595–608.
16. Sun TT. Altered Phenotype of cultured urothelial and other stratified epithelial cells: Implications for wound healing. Am J Physiol 2006;291(1): F9–21.
17. Wu XR, Manabe M, Yu J, et al. Large scale purification and immunolocalization of bovine uroplakins I, II, and III. Molecular markers of urothelial differentiation. J Biol Chem 1990;265:19170–9.
18. Deng FM, Liang FX, Tu L, et al. Uroplakin IIIb, a urothelial differentiation marker, dimerizes with uroplakin Ib as an early step of urothelial plaque assembly. J Cell Biol 2002;159:685–94.
19. Tu L, Sun TT, Kreibich G. Specific heterodimer formation is a prerequisite for uroplakins to exit from the endoplasmic reticulum. Mol Biol Cell 2002; 13:4221–30.
20. Wu XR, Medina JJ, Sun TT. Selective interactions of UPIa and UPIb, two members of the transmembrane 4 superfamily, with distinct single transmembrane-domained proteins in differentiated urothelial cells. J Biol Chem 1995;270:29752–9.
21. Christ GJ, Day NS, Santizo C, et al. Bladder instillation of "naked" hSlo/pcDNA3 ameliorates detrusor hyperactivity in obstructed rats in vivo. Urology 2001;57:111.
22. Boczko J, Tar M, Melman A, et al. Trypanosoma cruzi infection induced changes in the innervation, structure and function of the murine bladder. J Urol 2005;173:1784–8.
23. Pandita RK, Fujiwara M, Alm P, et al. Cystometric evaluation of bladder function in non-anesthetized mice with and without bladder outlet obstruction. J Urol 2000;164:1385–9.
24. Andersson KE, Arner A. Urinary bladder contraction and relaxation: physiology and pathophysiology. Physiol Rev 2004;84:935–86.