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Continuous Uroflow Cystometry in the Urethane-Anesthetized Mouse

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Abstract

Purpose—In vivo animal cystometry represents an accepted methodology for the study of lower urinary tract physiology. A particular advantage of the mouse model is the availability of genetically modified strains, offering the possibility of linking individual genes to relevant physiological events. However, small voided volumes complicate the ability to obtain reliable pressure-flow data by gravimetric methods, due to non-continuous drop formation and release during voiding. We investigated the feasibility of a simple non-gravimetric continuous urine collection system during cystometry under urethane anesthesia, and compared urethane-anesthetized with awake cystometry.

Methods—Cystometry was performed in awake and urethane-anesthetized female mice using a suprapubic tube. A simple, novel non-gravimetric method of urine collection was used in urethane-anesthetized animals to assess voided volume and permit flow rate calculations. Pressure and time-related variables were compared between groups.

Results—Voided urine collection appears to be complete and continuous in this model. Mean voided volume was 0.09 ± 0.020 ml, with an average flow rate of 0.029 ± 0.007 ml/sec. Urethane anesthesia delayed cystometric pressure/volume responses. However, micturition reflexes were intact and otherwise comparable between groups. Female mice void with pulsatile pressurization previously described in rats.

Conclusion—Suprapubic voiding cystometry using a simple and reliable urine collection method under urethane anesthesia is feasible in mice, permitting the integration of voided volumes with pressure and time data. The inclusion of volume and flow data enhances the usefulness of the mouse model for in vivo assessment of detrusor and potentially sphincteric performance.

Keywords

animal; models; urination; urodynamics

INTRODUCTION

Small animal models provide invaluable insights into lower urinary tract function and the pathophysiology of common clinical conditions. In vivo rodent cystometry is an accepted methodology for the study of the integrated determinants of lower urinary tract function, as well as the impact of injury, obstruction, and pharmacologic agents. The mouse model offers unique added value due to the broad availability of transgenic/gene knock-out strains. Such mouse strains have the potential to implicate individual genes in clinically relevant physiologic

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events, yet their broader use has been hindered by technological challenges of performing reliable lower urinary tract studies in mice.

Mouse cystometry has now been reported using both in vivo and ex vivo methodologies, involving natural filling, suprapubic and transurethral catheters, as well as anesthetized and awake animals. However, a full assessment of the hydraulics of micturition requires the simultaneous collection of pressure, time, and volume data. While this standard may be approached in human and larger animal models, mouse cystometry presents significant technical challenges as a result of the animal's small physical size and miniscule voided volumes (typically 0.2–0.3 ml per void). The volume of an individual drop depends upon the specific nature of the fluid and orifice, ranging between 0.05 and 0.1 ml.¹ Thus, a typical mouse void is two to six drops of urine. Moreover, placement of a catheter, particularly through the bladder dome (supra-pubic) as is typically done in mice, could be expected to further reduce this volume.

Voided volumes in mouse cystometry have been determined by either indirect assessment estimating spot size or through direct gravimetric methods. Typical gravimetric methods depend upon drop formation, free release from the meatus, and immediate unimpeded free-fall into a collection vessel on a load cell. Given the small number of drops per typical mouse void, any interference with this process may thus severely affect assessment of voiding volume and time. Incomplete drop collection has been reported to be problematic.² Furthermore, the formation of drops at the meatus quantizes the urine flow into samples of unknown volume and time variability. Therefore, as the number of drops per void is quite small, the precision of volume and flow measurement may be compromised. Methods involving post hoc analysis of spot size may be more reliable in assessing quantity, but do not permit any measurement of flow rate. Thus, commonly used collection methods are not suitable for per-void volume or flow determinations in mouse cystometry. Immobilization of the animal might allow alternative methods of urine collection, not dependent upon drop formation and release. Immobilizing restraint of an awake animal is likely to induce a significant stress artifact. As a result, cystometry with anesthesia represents a desirable alternative which permits such measurements in a still animal while avoiding animal stress.

With the above considerations in mind, we tested a simple urine collection system which allows continuous volume collection at the meatus, and therefore more precise voiding volume and time measurement. Since this method is dependent upon a still animal, urethane anesthesia was selected based upon its minimal impact on micturition.^{3–5} We therefore also sought to compare urethane-anesthesia cystometry with awake sub-acute cystometry.

METHODS

Cystometric Studies

Thirty-two adult female C57BL/6J mice, 15–37 g, were used. Prior to cystometric study, all animals were kept in standard housing cages with free access to food and water and normal 24 hr light cycling. The study protocol was approved by the University of Connecticut Health Center Animal Care Committee.

Catheters for cystometry were made of 15 cm lengths of PE50, placed under anesthesia into the dome of the bladder via an abdominal incision, secured with ligature, and tunneled subcutaneously. Catheter dimensions were chosen based upon preliminary investigation demonstrating that longer length and/or smaller diameter catheter tubing results in excessive damping of sudden pressure changes (data not shown). At the time of study, the catheter was connected to the cystometry tubing with a small compression fitting to avoid further constriction of the fluid line. A pressure transducer was placed in-line between a fluid pump

and the animal. Transducer outputs were digitized and recorded using WinDAQ software (DataQ, Dayton, OH). Following instrument calibration, room temperature normal saline was infused into the bladder at a rate of 0.025 ml/min. This instillation rate has been previously reported in mouse cystometry and is well within previously reported rates of 0.015 ml/min⁶ to 0.100 ml/min.^{7,8} All cystometries were conducted between 9 a.m. and 5 p.m. to minimize the effects of circadian changes in voiding function. After bladder instillation commenced, the system was allowed to stabilize to achieve a stable contraction/voiding pattern prior to data collection for analysis. Following cystometry, animals were euthanized with isoflurane.⁹

Urethane Cystometry

Animals were weighed and injected with urethane 1.2 g/kg in 1.5 ml saline into the neck scruff.¹⁰ When the animal was unresponsive to paw pinch (typically within 30–60 min of injection), the catheter was placed and exteriorized below the forelimb. Animals were positioned prone on a Lexan table with a 1.5 cm hole beneath the urethral meatus, allowing voiding urine to be collected in a 15 mm diameter, 3 ml collection cup loosely filled with tissue paper. The cup was suspended on a Grass FT03 force displacement transducer, and positioned with the tissue paper in very close proximity to the urethral meatus in order to collect and weigh voided urine at the meatus prior to droplet formation. Transducer output was amplified and sampled at 20 Hz, and recorded simultaneously with pressure data. Preliminary study was made using an infusion pump running at typical mouse flow rates into a length of PE50 as a urethral substitute, to test the collection method. This demonstrated our collection method resulted in an improved volume recording as compared to collection of fluid drops. Error versus nominal pump rates was equivalent or lower than with drop collection (Fig. 1).

Awake Cystometry

The catheter was placed under isoflurane (ca. 1.5%) inhalation anesthesia 2 days prior to cystometry. The catheter was exteriorized at, and coiled into, the neck scruff via a separate incision. Buprenorphine was administered for analgesia post-operatively. For cystometry, the catheter end was exteriorized under isoflurane (1.5%) anesthesia and the animals weighed. After fully awakening, cystometric study was commenced. During cystometry, the tubing/catheter was suspended above the animal to allow freedom of movement within a 15 cm × 20 cm × 15 cm open top Lexan box. The base was an open-weave wire (0.25 in.²) wire mesh, to allow observation of voiding from below.

Analysis

Voided volume and average flow rate were determined in the urethane-anesthetized animals. The voided volume was the difference in cumulative voided volume between the start and end of flow. The slope of the volume curve during flow (change of volume/change of time) was taken as the average flow rate. For cystometric analysis in all animals, three consecutive voiding contractions were chosen following attainment of a stable pattern. The parameters assessed were: voiding contraction pressure threshold (P_{ctx}), maximum voiding bladder pressure (P_{max}), voiding pressure change ($P_{delta} = P_{max} - P_{ctx}$), area under the bladder pressure curve (AUC), pressure drop rate (P_{loss} , waveform slope during the initial steep pressure decline of a voiding contraction); intervoid interval, compliance (change in pressure/change in volume, from the end of the previous void until onset of voiding contraction), and the amplitude and frequency of intermittent pulsatile high frequency oscillations (IPHFO) of bladder pressure during voiding.

Voiding volume parameters were not obtained in the awake cystometry. Voiding proved impossible to reliably assess as urine droplets frequently adhered to the perineum over the grid platform between voids. Therefore, voiding contractions were judged to be those of obviously greater amplitude and duration with typical rapid pressure declines, as shown in Figure 2. In

urethane cystometry, voiding contractions were those associated with a change in collected urine volume. During voiding, it was observed that prior to initiating flow, the perineum slightly descended to make contact with the tissue paper, then returned to its resting position just above the tissue in the post-void period. Therefore, voided volume was calculated as the change in volume registered during flow phase of the void, as animal movement caused small fluctuations in volume registered when not voiding.

Data were assessed for normality using Kolmogorov–Smirnov test, and compared between groups using Student *t* or Mann–Whitney test as appropriate. Categorical comparisons were made with Fischer exact test. For all comparisons, $P < 0.05$ was considered statistically significant.

RESULTS

A total of 17 awake and 15 urethane-anesthetized mice were prepared for cystometry. Of the awake mice, 8 were not analyzable; two due to technical failures (excessive movement artifact, catheter displacement), two did not develop consistent voiding patterns, and four did not develop analyzable voiding contractions. Of the urethane-anesthetized mice, five could not be analyzed; there was one technical failure (failed urine collection), three failed to develop consistent voiding patterns, and one died during cystometry. Thus, a total of 9 awake and 10 urethane cystometries were analyzed. There was no significant difference in success rate between the groups, nor in body weight.

Time expansion of the bladder pressure waveforms demonstrated IPHFO behavior during voiding, as shown in Figure 3. Rhabdosphincter-generated IPHFO contractions have been observed to accompany flow in rat voiding,¹¹ and are important for normal voiding function.¹² IPHFO activity was denoted by relatively high frequency, low amplitude bladder pressure oscillations associated with the pressure decline of a voiding contraction.

The mean voided volume in urethane-anesthetized mice was 0.09 ± 0.020 ml, with an average flow rate of 0.029 ± 0.007 ml/sec. The cystometric data from both groups are compared in Figure 4, presented as means with 95% CI. Contraction threshold pressure and maximum pressure were significantly higher in urethane animals than in awake mice. IPHFO frequency was higher in awake animals. Additionally, all urethane mice exhibited clear IPHFO activity associated with voiding flow, but five awake mice exhibited none, a significant difference ($P = 0.011$).

DISCUSSION

We present a method of continuous collection of small voided volumes in mice. The method is simple, employs commonly available materials, and appears to give reliable results. All voided volume is captured, thus, there is no risk of underestimation of per-void volumes due to meatal adherence of a terminal urine droplet. Transducer oscillation induced by sudden loading by falling drops is avoided, yielding a cleaner volume tracing during flow. The sampling rate of voided volume is not restricted to the rate of individual drop falls (i.e., 0.05–0.1 ml volume intervals). At a sampling rate of 20 Hz, as used in this study, at a flow rate of 0.029 ml/sec, the volume data is sampled at 0.0015 ml intervals, thus yielding a much more precise determinate of flow rate. As higher sampling rates may be selected in the analog–digital conversion prior to data acquisition, ultimate precision is limited to transducer specification. Finally, the physical arrangement precludes entrance of non-urine substances, thus, the recorded change in volume during voiding accurately reflects the per-void volume. Animals were observed to flex the meatus downwards during flow, and thus making contact with the tissue paper. It is possible that a capillary effect altered flow dynamics. However, flow rates

measured by this technique are in agreement with previously reported values using microsonographic assessment,^{13–15} suggesting that any artifact attributable to contact with the tissue paper is negligible.

Typical mouse void volume consists of only a few individual drops, probably precluding reliable and precise measurement of voided volumes and flow rates using standard gravimetric techniques. A methodology permitting voiding volume and rate determinations in addition to pressure data would enhance the usefulness of the mouse model for the in vivo assessment of detrusor and potentially sphincteric function. A suitable method requires some means of assessing flow prior to drop formation at the meatus. Micro-ultrasound has been employed to measure flow rates in male mice,^{13,14} but the instrumentation is not widely available, may require male animals, and may not be suitable for awake, unrestrained cystometry. Non-weight based electronic or imaging techniques might enable high sampling rates thereby achieving continuous flow data collection, however, their development has not been reported. We devised a simple collection system allowing continuous collection of the voided volume, and thus flow rates may be determined. However, this method requires that animals remain relatively immobile during the cystometry. An anesthetized animal requires minimal restraint and thus facilitates the collection method.

Urethane anesthesia was chosen based upon limited data regarding the effect of anesthetic agents on murine micturition. Inhalation agents, barbiturates and opioids significantly impair the micturition reflex.^{16,17} Ketamine/xylazine and urethane have been demonstrated to exert minimal impact on cystometry in rats, with the notable exception of a decreased bladder capacity, more pronounced with ketamine/xylazine than with urethane.^{5,16} The impact of urethane on mouse voiding relative to voiding pressure/volume/time parameters has not been previously evaluated. This study allowed assessment of the effect of urethane only on timing and pressure variables.

Considerable artifact was demonstrated in the awake cystometry group. We employed PE50 tubing, rather than the more flexible PE10 commonly used for mouse cystometry. This was on the basis of preliminary testing demonstrating significant impairment of pressure transmission using 10–15 cm lengths of PE10. It is possible that the relatively stiff and bulky (compared to the mouse bladder) PE50 tubing-induced bladder irritation and/or perturbed normal bladder mechanics during filling and voiding, accounting for greater artifact in the cystometric tracings and possibly a seemingly high rate of uninterpretable experiments. The higher thresholds observed in the urethane group may obscure catheter-induced perturbations.

With these limitations in mind, the most significant differences between awake and urethane-anesthetized cystometry were observed with $P_{\max}$ and P_{ctx} , although neither P_{delta} nor AUC was significantly different. The impact of urethane on murine voiding thus appears to be one of a delayed voiding pressure (and therefore volume, as bladder compliance in both groups was equivalent) thresholds. Once activated, however, the relative magnitude of the voiding contraction is not changed. This and the absence of other significant differences between groups suggests that the voiding threshold delay due to urethane may be attributable to suppressed sensory function and not an alteration of the central/efferent aspects of voiding. Voided volumes in the urethane group were also lower than expected. Possible causes include altered dynamics or bladder irritability related to the catheter (as above) and/or reflexic inhibition of normal voiding due to suppressed sensory function. We did not attempt to assess post-void residuals and therefore cannot comment on relative voiding efficiencies of awake versus urethane anesthesia.

Only four of nine animals in the awake group demonstrated clear IPHFO contractions during voiding, whereas all urethane animals did, a significant difference. This may be due to the

higher voiding pressures in the urethane group, as these pressures would be transmitted to the urethra. Urethral afferents are pressure-sensitive,¹⁸ and the afferent limb of the IPHFO response originates in the urethra.^{12,19} IPHFO activity in normal rat voiding occurs at a rhabdosphincter contraction frequency of 8–10 Hz,¹² however, we observed lower mean frequencies, presumably more optimal for the flow characteristics of the smaller mouse bladder and outlet.

CONCLUSIONS

The benefit of a still, unrestrained animal and the consequent ability to obtain continuous volume and flow data with relatively simple methods suggests the usefulness of this urethane-anesthetized, acute suprapubic catheter, mouse cystometry model. Since reliable pressure/flow data is obtainable, this model permits a more complete cystometric assessment of the relative contribution of detrusor and outlet function to overall lower urinary tract performance. Urethane altered the micturition cycle in a predictable manner, consistent with its anesthetic effect.

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Fig. 1.

Testing of urine collection method. Volume recording at nominal infusion rates of 80, 100, and 120 ml/hr (0.022, 0.028, 0.033 ml/sec). Left graph demonstrates tracing of collection of free falling drops (distance, 2 cm) at 100 ml/hr. Right graph demonstrates tracing of paper-damped collection at 100 ml/hr, as used in urethane cystometry. Error is reported as percentage deviation from nominal infusion rate.

Fig. 2. Examples of voiding tracings in awake (top) and urethane-anesthetized (bottom) animal, acute suprapubic catheterization. Awake tracing is bladder pressure (cm of water) only. Voiding contractions are denoted by arrows in awake tracing (see text). Urethane tracing shows bladder pressure (cm of water, top) and volume voided (ml, bottom). Flow rate is the slope of volume tracing during voiding contraction pressure drop. Each horizontal division = 10 sec.

Fig. 3.

Example of time-expanded bladder pressure tracing during voiding. Boxed area illustrates intermittent pulsatile high frequency oscillations (IPHFO) activity during flow. Pressure (cm of water, top tracing) rises per-flow, and flow is accompanied by IPHFO pressure fluctuation. Upon flow termination, pressure rises isometrically prior to dissipation of voiding contraction. Bottom trace is cumulative voided volume (ml). Each horizontal division = 0.5 sec.

Fig. 4.

Pressure, time and wall performance data, awake versus urethane murine cystometry. P_{max} is the maximum voiding bladder pressure (cm of water), P_{ctx} the voiding contraction pressure threshold (cm of water), P_{delta} the contraction pressure change ($P_{max} - P_{ctx}$) (cm of water), P_{loss} the slope of maximal initial voiding pressure decline (cm of water/sec), IPHFO Hz the IPHFO contraction frequency (Hz), P_{ipho} the IPHFO contraction pressure rise (cm of water), Interval the start-to-start interval between voiding contractions (minutes), AUC the area under voiding contraction curve (cm of water sec), Compliance the dP/dV (cm of water/ml). Shown are means with 95% CI bars. * $P = 0.007$, ** $P = 0.024$, *** $P = 0.0069$.