Adjustable passive stiffness in mouse bladder: regulated by Rho kinase and elevated following partial bladder outlet obstruction

John E. Speich,1 Jordan B. Southern,2 Sheree Henderson,3 Cameron W. Wilson,2 Adam P. Klausner,2 and Paul H. Ratz3

Departments of 1Mechanical and Nuclear Engineering, 2Surgery, 3Biochemistry & Molecular Biology and Pediatrics, Virginia Commonwealth University, Richmond, Virginia

Submitted 2 April 2011; accepted in final form 22 December 2011

Speich JE, Southern JB, Henderson S, Wilson CW, Klausner AP, Ratz PH. Adjustable passive stiffness in mouse bladder: regulated by Rho kinase and elevated following partial bladder outlet obstruction. Am J Physiol Renal Physiol 302: F967–F976, 2012. First published December 28, 2011; doi:10.1152/ajprenal.00177.2011.—Detrusor smooth muscle (DSM) contributes to bladder wall tension during filling, and bladder wall deformation affects the signaling system that leads to urgency. The length-passive tension (L-Tp) relationship in rabbit DSM can adapt with length changes over time and exhibits adjustable passive stiffness (APS) characterized by a L-Tp curve that is a function of both activation and strain history. Muscle activation with KCl, carbachol (CCh), or prostaglandin E2 at short muscle lengths can increase APS that is revealed by elevated pseudo-steady-state Tp that follows by complete washout of the activating stimulus can generate APS that is revealed by elevated pseudo-steady-state Tp at longer lengths compared with prior Tp measurements at those lengths, and APS generation is inhibited by the Rho Kinase (ROCK) inhibitor H-1152. In the current study, mouse bladder strips exhibited both KCl- and CCh-induced APS. Whole mouse bladders demonstrated APS which was measured as an increase in pressure during passive filling in calcium-free solution following CCh precontraction compared with pressure during filling without precontraction. In addition, CCh-induced APS in whole mouse bladder was inhibited by H-1152, indicating that ROCK activity may regulate bladder compliance during filling. Furthermore, APS in whole mouse bladder was elevated 2 wk after partial bladder outlet obstruction, suggesting that APS may be relevant in diseases affecting bladder mechanics. The presence of APS in mouse bladder will permit future studies of APS regulatory pathways and potential alterations of APS in disease models using knockout transgenetic mice.

DURING ITS FILLING phase, the bladder accommodates and stores urine under low intravesical pressures. To maintain low pressures over a broad volume range, the bladder wall must be compliant as it slowly undergoes significant deformation. On the other hand, a certain degree of bladder wall stiffness during filling is critical because localized stretching of the bladder wall activates the mechanosensitive afferent nerve fibers responsible for urgency (14), and overactive bladder (OAB), a common condition involving urgency during filling, affects ~17% of the adult population worldwide (33).

The tension produced by detrusor smooth muscle (DSM) in the bladder wall has both active and passive components. Until the 1990s, the active and passive length-tension relationships of smooth muscles were believed to be static, as in skeletal muscle, with a single maximum active tension (Tₐ) and a single passive tension (Tp) value at each muscle length. However, recent studies on airway smooth muscle (4, 8, 9, 20, 24, 40, 52), vascular smooth muscle (6, 39, 49), and DSM (2, 44, 46) demonstrated that these smooth muscles have dynamic length-tension relationships that adapt based on activation and strain history. In rabbit DSM, the length-passive tension (L-Tp) curve exhibits adjustable passive stiffness (APS) characterized by a passive curve that can be shifted along the length axis as a function of activation and strain history (1, 37, 44–47). More specifically, maximal KCl- or carbachol (CCh)-induced contractions or smaller amplitude CCh- or prostaglandin E₂ (PGE₂)-induced rhythmic contractions at short muscle lengths followed by complete washout of the activating stimulus can generate APS that is revealed by elevated pseudo-steady-state Tp at longer lengths compared with prior Tp measurements at those lengths. That is, these forms of muscle activation shift the subsequent L-Tp curve to the left, and the muscle length during activation before generation of the L-Tp curve is inversely proportional to the amount of APS generated (1, 45, 46).

Moreover, APS generation in rabbit DSM is inhibited by the selective Rho kinase (ROCK) inhibitors H-1152 and Y-27632 (37, 45), the actin polymerization inhibitor latrunculin-B (37), and the selective myosin II inhibitor blebbistatin (37), providing evidence that actomyosin crossbridges regulated by ROCK are responsible for APS.

Partial bladder outlet obstruction (PBOO) is a clinical condition resulting from benign prostatic hyperplasia that has been shown to produce changes in bladder compliance (31) and detrusor overactivity in humans (35) and animal models (30, 38). Whether APS is altered due to PBOO remains to be determined. The present study was designed to determine whether mouse bladder strips and whole mouse bladders exhibit APS, whether APS in mouse bladder is regulated by ROCK, and whether PBOO affects APS in mouse bladder.

METHODS

Tissue preparation. All experiments involving animals were conducted with the appropriate animal welfare regulations and guidelines and were approved by the Virginia Commonwealth University Institutional Animal Care and Use Committee. Whole bladders were obtained from adolescent and adult mice euthanized by CO₂ inhalation. Some mouse bladders were provided by the George M. O’Brien Urology Research Center at the University of Pennsylvania 2 wk after they had undergone a PBOO procedure. PBOO was performed via partial urethral ligation using established protocols (3) in which 7–0 polypropylene suture was placed around the bladder neck and tied down over a 24-gauge needle (0.56-mm outer diameter) that was then removed. Male mice were used in the PBOO study because PBOO can occur in men with benign prostatic hyperplasia. Adult female mice...
were used in all but one of the other experiments so that they could be compared with our previous APS studies that used adult female rabbits (1, 37, 44–47).

All bladders were washed, cleaned of adhering tissues, including fat and serosa, and stored in cold (0–4°C) modified physiologic salt solution. For muscle strip studies, bladders were cut from the urethral opening along each lateral surface toward the dome and the urethra-lum was removed. The dome was not cut, allowing the full bladder to be opened like a clam shell, with both halves of the urethra now at opposing ends and the dome at the center of the bladder strip. For one experiment, each full-bladder strip was cut in half vertically to produce two half-bladder strips. Each strip was attached at the urethral ends to muscle clips and placed in a muscle chamber for length adjustments and tension measurements.

**Solutions.** Modified physiological salt solution (PSS) was composed of 140 mM NaCl, 4.7 mM KCl, 1.2 mM MgSO4, 1.6 mM CaCl2, 1.2 mM Na2HPO4, 2.0 mM morpholino propane sulfonic acid (adjusted to pH 7.4 at either 0 or 37°C, as appropriate), 0.02 mM Na2 ethylenediamine tetraacetate acid, and 5.6 mM dextrose. Muscle contractions were induced with PSS modified to include 110 mM KCl, substituted isosmotically for NaCl, (KPSS). A Ca2+-free solution (0-Ca) was PSS without CaCl2.

**Bladder strip setup.** Each mouse bladder strip was secured by small clips such that the initial (cold) zero preload length was ~2–5 mm. One end was attached to a micrometer to permit manual length adjustments and the other end was attached to a force transducer (159901a, Radnoti Glass Technology, Monrovia, CA) to record isometric tension. Each tissue was allowed to gradually warm to 37°C in aerated PSS in a water-jacketed tissue bath for a 45- to 60-min equilibration period.

**Reference tension and length determination for bladder strips.** For each bladder strip, a stepwise L-T protocol was applied to identify a peak tension value and the corresponding muscle length on the L-T max curve and these values were used as the reference tension (Tref) and length (Lref). In short, each bladder strip was incubated for 2 min in 0-Ca, stretched to the next length and allowed to stress-relax for 2 min, washed for 3 min in PSS, and stimulated with KCl to produce a maximum contraction. Following washout of KCl to cause complete relaxation, the protocol was repeated until Tref was identified. For each muscle length, Tp was measured at the end of the stress-relaxation period, and Tref was calculated as the peak isometric contraction induced by KCl minus Tp. 0-Ca was used to prevent Ca2+-dependent cross bridge activation. To account for potential tension development while tissues were incubated in PSS after the 4-min incubation in 0-Ca and before stimulation with KCl, Tp was taken as the lowest value either in PSS or 0-Ca, as previously described (44).

PSS was added to ensure “reloading” of the tissue with Ca2+ to ensure the KCl produced a brisk tension increase. Note that because the L-Ta relationship in DSM exhibits length adaptation (2, 44), Tref and Lref do not represent static optimal tension and length values associated with skeletal muscles (23). For the first APS protocol described in the next section, all length steps were 1 mm, and the maximum length (Lmax) to which each bladder strip was stretched was 1 mm beyond Lref. For the second protocol, some steps of less than 1 mm (mean = 0.76 mm) were taken to limit peak Tp during the stretch to less than the total tension during the contraction at the previous length.

**APS measurement protocols for bladder strips.** To quantify KCl-induced APS, half-bladder strips from adolescent mice (6- to 7-wk-old; 2 female and 2 male) were subjected to three cycles of a three-step loading/unloading protocol (Fig. 1A). Tissues undergoing this loading and unloading protocol to measure APS were always maintained in a 0-Ca solution to ensure that Ca2+-dependent cross bridge activation was not a contributing factor to the Tp measured. Three-step loading involved increasing muscle length to 80, 90, and 95% of Lmax and three-step unloading involved decreasing muscle length to 95, 90, and 80% of Lmax (Fig. 1A). To minimize time for stress relaxation during the loading phase that can take hours to reach a steady state (47), Tp measurements were performed after short releases (e.g., 82 to 80% Lmax, Fig. 1A, open bars). To allow a step-down to 95% Lmax each tissue was stepped-up to 100% Lmax before stepping down to 95% Lmax (Fig. 1A).

Each of the three cycles of the three-step loading-unloading protocol was performed following a particular pretreatment. The first pretreatment was to incubate in 0-Ca for 11 min at 50% Lmax. The second pretreatment was to contract twice using KCl at 50% Lmax (timing: 3 min PSS, 1 min KCl, 3 min PSS, 1 min KCl, 3 min 0-Ca). The third pretreatment was to incubate in PSS at 50% Lmax (timing: 8 min PSS, 3 min 0-Ca). During each cycle, isometric Tp was measured at the steady state following the step-change in length to 80, 90, and 95% Lmax during both loading and unloading (Fig. 1A, filled bars, and B).

A similar protocol was designed to measure CCh-induced APS (Fig. 2); although in this protocol, the length changes were to 70, 80, and 90% of Lmax rather than to 80, 90, and 95% of Lmax. Full-bladder strips from young adult (10–11 wk old) female mice were subjected to four cycles of this protocol while incubated in 0-Ca solution. The first and third iterations were performed after two 10-μM CCh-inducted contractions at 50% Lmax (timing: 3 min PSS, 1 min CCh, 3 min PSS, 1 min CCh, 3 min 0-Ca), and the second and fourth iterations were performed after incubation in 0-Ca for 11 min at 50% Lmax. Based on prior studies of APS in rabbit DSM (1, 37, 45–47), we expected to find greater Tp during the loading phases of the these two protocols in which tissues were precontracted with KCl or CCh.

**Whole bladder setup.** Urethra openings of bladders were secured to a blunted 18-gauge needle using 0.04-mm tungsten wire and connected to a syringe pump (55–4143, Harvard Apparatus, Holliston, MA) to control bladder volume and a pressure transducer (Radnoti Glass Technology) to measure vesicular pressures. Empty bladders were suspended by the needle in aerated PSS at 37°C in a water-jacketed tissue bath and equilibrated for 45–60 min.

**Reference pressure and volume determination.** Isovolumetric passive and active pressures were measured at a minimum of four 10- to 20-μl increments (Fig. 3) until a peak active pressure value was identified. At each volume, bladders were relaxed in PSS for 3 min and then passive pressure was measured, incubated in KPSS for 1 min to induce a maximal contraction, relaxed in PSS for 3 min, and then filled to the next volume in ~10–20 s. Active pressure at each volume was calculated by subtracting passive pressure from the peak total KCl-induced pressure at that volume. The data from each bladder were fitted to a parabola, and the peak active pressure and corresponding volume were used as the reference pressure (Pref) and volume (Vref) for that bladder.

**General APS measurement protocol for mouse bladders.** Following the determination of Vref, each mouse bladder was subjected to several constant-rate fill-empty cycles between 10 and 20% Vref and 100% Vref. As in the tissue strip protocol in which APS was measured by applying loading-unloading cycles, application of each fill-empty cycle was done while the bladders were incubated in a 0-Ca solution to ensure elimination of Ca2+-dependent crossbridge activation. Total filling time was 10 min and total emptying time was 1 min. The first fill-empty cycle after identification of Vref was discarded as a preconditioning cycle. Before the filling phase of each cycle, bladders were either incubated in 0-Ca for 11 min or stimulated with CCh or KCl. Based on prior studies of APS in rabbit DSM (1, 37, 45–47), we expected to find greater passive filling pressures after bladders were precontracted with CCh or KCl. Filling pressure due to APS was calculated as the difference between the passive filling pressure following preconstriction with CCh or KCl and the filling pressure following incubation in 0-Ca with no preconstriction (Fig. 4A). Both the pressure due to APS at 40% Vref and the peak pressure due to APS during filling from 10 to 20% Vref to 100% Vref were analyzed.

Filling pressure during cystometry is fill-rate dependent (26). We used 10-min fills to approximate typical infusion rates used in human cystometry (30–60 ml/min will fill a human bladder to a normal
capacity of 400 ml in 7–13 min) (28, 42). Mice void their urine approximately once per hour (11); therefore, to demonstrate the difference between 10-min passive filling and much slower, more physiologic 55-min filling, one bladder was filled at each rate with and without precontraction with CCh (Fig. 4B). As expected, the faster fill rate produced much greater pressures (Fig. 4B). Notably, for the present study, APS was present at both filling rates.

**Effects of 10 μM staurosporine, 1.0 μM H-1152, and deionized water on APS.** Following the determination of Vref, bladders from adult female mice (11–26 wk old) were divided into three groups; control, staurosporine (STP), and H-1152, and subjected to four constant-rate fill-empty cycles between 10% Vref and 100% Vref as described in the general APS measurement protocol in the previous section. Before the filling phase of the first and third cycles, bladders from all three groups were stimulated with 10 μM CCh (timing: 3 min PSS, 5 min CCh in PSS, 3 min 0-Ca), and before the second and fourth cycles bladders from all three groups were incubated in 0-Ca for 11 min.

APS development in rabbit DSM is inhibited by the general serine and threonine protein kinase inhibitor STP (1) and by the ROCK inhibitor H-1152 (37). To determine whether APS in whole mouse bladder was also regulated by these kinases, 10 μM STP and 1.0 μM H-1152 were added to the STP and H-1152 groups, respectively, during both the incubation period in PSS and the CCh stimulation period before only the third filling cycle.

Finally, a prior study showed that APS was abolished when rabbit DSM strips were lysed by incubation in deionized water (1). To quantify this effect in mouse whole bladder, some bladders were subjected to two additional fill-empty cycles. These cycles were completed in deionized water following a 10-min incubation period in deionized water. We expected to find reduced passive filling pressure in nonviable tissues incubated in deionized water compared with filling pressure in viable tissues incubated in 0-Ca.

**Effect of PBOO on APS.** Bladders were harvested from two groups of adult (8–9 wk old) male mice: a PBOO group in which bladders were harvested 2 wk after the PBOO procedure (6 mice, 3 Swiss-Webster and 3 C57BL/6J) and a control group in which the PBOO procedure was not performed (7 mice, 3 Swiss-Webster and 4 C57BL/6J, 1 of which underwent a sham PBOO procedure). PBOO bladders from two strains of mice were used because those were the bladders available from the George M. O’Brien Urology Research Center at the University of Pennsylvania at the time of the study. We used a similar distribution of control bladders from the two strains.

Following the determination of Vref, bladders were subjected to three fill-empty cycles as described in the general APS measurement protocol section. The fill-empty volume range was 20% Vref to 100% Vref, and before the filling phase of each cycle, bladders were either incubated in 0-Ca for 11 min, stimulated twice with KCl (timing: 3 min PSS, 1 min KPSS, 3 min PSS, 1 min KPSS, 3 min 0-Ca), or stimulated with 1 μM CCh (timing: 3 min PSS, 5 min CCh in PSS, 3

---

**Fig. 1.** A: loading/unloading protocol for measuring passive tension (T_p) due to KCl-induced adjustable passive stiffness (APS) in mouse bladder strips. B: typical T_p tracings. C: length-passive tension (L-T_p) loading and unloading curves in Ca^{2+}-free solution (0-Ca) following precontraction with KCl at 50% L_{max} [T_p values normalized to reference tension (Tref), n = 4]. D–F: T_p at 80, 90, and 95% L_{max} normalized to T_p during loading at that length following KCl precontraction (T_p/T_p_load_KCl; means ± SE, * indicates value significantly less than 1.0, and Ω indicates unloading value significantly less than the corresponding loading value, P < 0.025, n = 4).
min 0-Ca). Following the pressure-volume (P-V) experiments, the wet weight of each bladder was measured.

Statistical analyses. Analyses were performed using Prism (5.0, GraphPad Software, La Jolla, CA) or Excel (2007, Microsoft, Redmond, WA). A t-test with the Bonferroni correction was used to determine significant differences when comparing one group to two other groups, and a one-way ANOVA with the post hoc Student-Newman-Keuls test was used when comparing more than two groups. The null hypothesis was rejected at $P < 0.05$. The sample size ($n$ value) refers to the number of animals (bladders) and not the number of tissues.

RESULTS

APS in mouse bladder strips. For the mouse bladder strips subjected to the protocol shown in Fig. 1A, the initial lengths were $\sim 2$ mm, $L_{\text{ref}}$ was $5.25 \pm 0.85$ mm, $L_{\text{max}}$ was $6.25 \pm 0.85$ mm and $T_{\text{ref}}$ was $24.0 \pm 1.7$ mN (means $\pm$ SE, $n = 4$). These strips produced pressure tracings similar to the example shown in Fig. 1B and the normalized $L_{\text{ref}}$-$T_p$ curves in Fig. 1C. $T_p$ values at 80, 90, and 95% $L_{\text{max}}$ were normalized to $T_{\text{ref}}$ at that length following CCh precontraction ($T_p/T_{\text{ref}}$, CChA: * indicates value significantly less than 1.0, and $\Omega$ indicates an unloading value significantly less than the corresponding loading value, $P < 0.025, n = 3$).

Fig. 2. A: loading/unloading protocol for measuring $T_p$ due to carbachol (CCh)-induced APS in mouse bladder strips. B: $L_{\text{ref}}$-$T_p$ loading and unloading curves following 10-$\mu$M CCh precontractions (CChA and CChB) or no precontractions (incubation in 0-Ca, 0-CaA, and 0-CaB) at 50% $L_{\text{max}}$ (means $\pm$ SE, $n = 3$).

Fig. 3. Passive and active pressure-volume curves for female mouse bladders (means $\pm$ SE for pressure and volume, * indicates passive pressure value significantly greater than passive pressure at 20 $\mu$L, and $\psi$ indicates that active pressure at 72.5 $\mu$L was significantly less than at 59 $\mu$L, paired t-test, $P < 0.05, n = 10$).

Fig. 4. A: passive filling pressure in 0-Ca during 10-min fills with (stiff) and without (compliant) a 1-$\mu$M CCh precontraction at 10% $V_{\text{ref}}$. The pressure difference ($P_{\text{APS}} = \text{stiff} - \text{compliant}$) was attributed to APS. B: passive filling pressure during 10- and 55-min fills with and without a 1-$\mu$M CCh precontraction.
measured during the loading phase at that length following precon- 
traction with KCl (Figs. 1, D–F, $T_p/T_{p_{load, KCl}}$). Compared 
with incubation in 0-Ca or PSS without a precontraction, 
precontraction with KCl at 50% $L_{max}$ induced significantly 
greater pseudo-steady-state $T_p$ at each length during passive 
loading in 0-Ca (Fig. 1, C–F, loading after KCl compared with 
loading after 0-Ca or PSS, *).

Notably, these tissues showed significantly less pseudo- 
steady-state $T_p$ in 0-Ca during the unloading phase of the protocol 
compared with the loading phase for all tissues (those 
that were precontracted with KCl and those that were not 
precontracted and incubated in PSS and in 0-Ca; Fig. 1, C–F, 
unloading curves compared with loading curves, $\Omega$). Thus, the 
$L-T_p$ relationship for mouse bladder strips during passive 
loading and unloading was both strain-history and activation-
history dependent, revealing the presence of APS.

For the mouse bladder strips subjected to the protocol shown 
in Fig. 2A, the initial length was 5.02 ± 0.037 mm, $L_{ref}$ was 
9.72 ± 0.61 mm, $L_{max}$ was 10.23 ± 0.65 mm, and $T_{ref}$ was 
14.9 ± 4.5 mm (means ± SE, $n = 3$). Similarly, mouse bladder 
strips produced the normalized $L-T_p$ curves in Fig. 2B when 
subjected to four cycles of the protocol shown in Fig. 2A which 
alternated between precontraction with 10 $\mu$M CCh at 50% 
$L_{max}$ (CChA and CChB) and incubation in 0-Ca without precon-
traction at 50% $L_{max}$ (0-CaA and 0-CaB). As in the previous 
experiment with KCl, tissues exhibited significantly less pseu-
do-steady-state $T_p$ at each length during passive loading in 
0-Ca when they were not precontracted compared with when 
they were precontracted with CCh at 50% $L_{max}$ (Fig. 2, C–E, 
0-CaA and 0-CaB compared with CChA and CChB, *), and the 
results were repeatable for multiple cycles with and without 
precontraction (Fig. 2, C–E, compare CChA and 0-CaA with 
CChB and 0-CaB). Tissues also showed significantly less pseudo-
steady-state $T_p$ in 0-Ca at 70, 80, and 90% $L_{max}$ during the 
unloading phase of the protocol compared with the loading 
phase for both tissues that were precontracted with CCh and 
those that were not precontracted (Fig. 2, C–E, unloading 
curves compared with loading curves, $\Omega$).

APS in mouse whole bladder. Although whole bladders from 
the female mice produced a relatively flat passive P-V curve, 
passive pressure did increase with increasing volumes beyond 
40 $\mu$l (Fig. 3). The active P-V curve (Fig. 3) displayed 
ascending and descending limbs corresponding to the limbs 
exhibited by the active $L-T$ curve for isolated strips of DSM (2, 
44, 50, 51). In 10 of the 14 female mouse bladders used in the 
present study, $P_{ref}$ was identified by measuring pressures at 
four volumes, and the corresponding passive and active P-V 
curves are shown in Fig. 3. The active P-V curve has relatively 
large error bars because for some bladders the peak pressure 
was at the second measurement and the third measurement 
was on the descending limb and for other bladders the second 
measurement was on the ascending limb and the peak was at the 
third measurement. For the remaining four bladders, 
identification of $P_{ref}$ required inclusion of pressure measure-
ments at additional volumes. For the complete group of 14 
bladders, $P_{ref}$ was 43.7 ± 3.6 mmHg (59.4 ± 4.9 cmH2O) 
and $V_{ref}$ was 58.0 ± 4.5 $\mu$l ($n = 14$).

Passive pressures measured in tissues incubated in 0-Ca 
during 10-min fills from 10% $V_{ref}$ to 100% $V_{ref}$ were typically 
greater when whole bladders were precontracted with 10 $\mu$M 
CCh at 10% $V_{ref}$ compared with when they were not precon-
tracted and incubated in 0-Ca at 10% $V_{ref}$, as shown in the 
example P-V curves in Fig. 4A. In this example, the bladder 
was stiffer when precontracted with CCh and more compliant 
when it was not precontracted. Throughout the present study, 
passive pressure due to APS ($P_{APS}$) was calculated as the 
pressure difference between the fill following a precontraction 
(stiff) and the fill without a precontraction (compliant), as 
illustrated in Fig. 4A. As mentioned in the METHODS section, it 
is important to note that $P_{APS}$ is evident at both the very slow, 
physiological filling rate (Fig. 4B, lower 2 curves), and the 
more rapid filling rate used in the present study for our 
experimental analyses (Fig. 4B, upper 2 curves) that corre-
sponds closely to the filling rate used in human cystometry. 
Because slow filling was for 55 min, the data indicate that $P_{APS}$ 
can be preserved even in tissues incubated for nearly 1 h in 
0-Ca.

Passive pressure at 40% $V_{ref}$ was significantly greater in 
bladders that were filled from 10% to 100% $V_{ref}$ while incu-
bated in a 0-Ca solution after they had been precontracted with 
10 $\mu$M CCh than in control bladders that had not been 
precontracted but were simply pretreated by incubation in 0-Ca 
for the same pretreatment duration (Fig. 5, A–B, 0-CaA com-
pared with CChA, *). This characteristic was repeatable (Fig. 5, 
A–B, 0-CaB compared with CChB, Control, *). Normalized 
passive pressure and $P_{APS}$ at 40% $V_{ref}$ were both significantly 
less when the CCh-induced precontraction was inhibited with 
ether 10 $\mu$M STP or 1 $\mu$M H-1152 (Fig. 5, B–C, *).

In addition to analyses of the passive pressure caused by 
the generation of APS at the single volume of 40% $V_{ref}$, the peak 
APS during filling from 10% to 100% $V_{ref}$ was also quantified. 
Peak $P_{APS}$ was 4.85 ± 0.56 mmHg for the first pair of fill-empty 
cycles, and this value significantly increased to 6.39 ± 0.67 
mmHg for the second pair (Fig. 6A, $\Psi$). As a fraction of $P_{ref}$, 
$P_{APS}$ was 0.12 ± 0.01 for the first pair of fill-empty cycles, and 
this value significantly increased to 0.16 ± 0.01 for the second 
pair (Fig. 6B, $\Psi$). Furthermore, normalized peak $P_{APS}$ was 
significantly less when the CCh-induced precontraction was 
inhibited with either 10 $\mu$M STP or 1 $\mu$M H-1152 (Fig. 6C, *).

Together, these data indicate that the passive P-V relationship 
in mouse bladder is a function of activation history, revealing 
the presence of APS in whole mouse bladder. Moreover, these 
data indicate that APS generation in mouse bladder is due to an 
active process involving STP- and H-1152-sensitive regulation 
and not by passive viscoelastic effects. Whole bladders incu-
bated in deionized water for 10 min at 10% $L_{max}$ and during a 
fill-empty cycle showed a significant decrease in passive filling 
pressure at 40% $V_{ref}$ compared with the filling pressure measured 
when tissues were incubated in 0-Ca at that volume (Fig. 7, $\Omega$). 
Because deionized water will destroy cellular integrity (21), 
this finding supports the contention that DSM cellular pro-
teins play a major role in the establishment of $T_p$ at 40%, 
and these data are consistent with our prior study in rabbit 
DSM strips (1).

APS in whole bladders from PBOO mice. Passive and active 
P-V curves for male PBOO and control mouse bladders are 
presented in Fig. 8A. The P-V curves for two of the PBOO 
bladders were substantially different from the other four, and 
therefore the PBOO data were divided into two groups that 
were labeled “compensated PBOO” and “decompensated 
PBOO” (3, 12, 48). For each bladder in the compensated 
PBOO group, $V_{ref}$ was less than 100 $\mu$l, and for both bladders
in the decompensated PBOO group, V_{ref} was greater than 165 µL. V_{ref} for the control and compensated PBOO groups was not different (Fig. 8B); however, the corresponding peak active pressure, P_{ref}, was reduced for the compensated PBOO group (Fig. 8C). The active P-V curve for the compensated PBOO group was lower than the control curve and this difference approached statistical significance at volumes of 80 µL and greater. The active P-V curve for the decompensated PBOO group was lower than the curve for the compensated PBOO group (Fig. 8A) as previously reported (3). Passive pressure for the compensated PBOO group was statistically greater than the control group at volumes of 20 µL and greater; however, the decompensated PBOO group did not show a similar trend. Wet weights for the bladders varied substantially as shown in Fig. 8D, with the compensated PBOO bladders showing a trend toward a greater average weight, as has been previously reported (3).

KCl-induced peak P_{APS} was elevated in the compensated PBOO group compared with the control group (Fig. 9A) and CCh-induced peak P_{APS} showed a similar trend. Furthermore, when normalized to P_{ref}/weight, both KCl- and CCh-induced P_{APS} were elevated in the compensated PBOO group (Fig. 9B), and the two decompensated PBOO bladders showed the same trend.

**DISCUSSION**

**Contribution of APS in mouse bladder.** The L–T_{p} relationship for mouse bladder strips during passive loading and unloading was both strain-history and activation-history dependent (Figs. 1 and 2), revealing the presence of APS in mouse bladder strips. We attribute the increase above the control value in T_{p} witnessed during the passive loading of mouse bladder strips following precontraction with KCl or CCh at muscle lengths shorter than 50% L_{max} (Figs. 1, D–F, and 2, C–E) to the establishment of long-lasting cross-links induced by the contractile stimuli (1, 37, 45–47). Our data support the contention that the additional T_{p} displayed during loading compared with unloading (Figs. 1, D–F, and 2, C–E) was due to a combination of APS and viscoelastic stiffness (37). APS was lost by the strain softening that occurred during stretches (e.g., to L_{max}) (1, 37, 45–47). As we previously reported for rabbit DSM (37, 47), viscoelastic stiffness of mouse bladder was manifested as a component of stress relaxation that ap-

![Image](http://ajprenal.physiology.org/DownloadedFrom/1022033.04.2.17)

---

**Fig. 5.** Passive filling pressure data at 40% reference volume (V_{ref}) for 3 groups of bladders: control, staurosporine (STP), and H-1152. Each group underwent 4 passive filling cycles from 10% to 100% V_{ref}, with the 1st and 3rd following precontraction with 10 µM CCh and the 2nd and 4th following incubation in 0-Ca with no precontraction (CCh_{A}, 0-Ca_{A}, CCh_{B}, and 0-Ca_{B}). A: pressure in control bladders at 40% V_{ref} during each of the 4 filling cycles (means ± SE, * indicates 0-Ca value significantly less than the prior CCh value, P < 0.05, n = 6). B: effects of 10 µM STP and 1.0 µM H-1152 during CCh stimulation on the subsequent normalized passive pressure at 40% V_{ref} during the 3rd fill-empty cycle (CCh_{B} ± drug; * indicates values significantly less than 1.0, P < 0.05, n = 6 control, n = 3 STP and H1152). C: effects of 10 µM STP and 1.0 µM H-1152 during CCh stimulation before the 3rd cycle on normalized passive pressure attributed to APS (P_{APS}) at 40% V_{ref} during the 3rd passive filling cycle (P_{APS,A} = CCh_{A} − 0-Ca_{A}, P_{APS,B} ± drug = CCh_{B} ± drug − 0-Ca_{B}, Ω and * indicate values significantly greater than or less than 1.0, respectively, P < 0.05, n = 6 control, n = 3 STP and H1152).

---

**Fig. 6.** A: peak pressure attributed to APS during 4 passive bladder filling cycles from 10% to 100% V_{ref}, with the 1st and 3rd following precontraction with 10 µM CCh and the 2nd and 4th following incubation in 0-Ca with no precontraction (CCh_{A}, 0-Ca_{A}, CCh_{B}, and 0-Ca_{B}), with P_{APS,A} = CCh_{A} − 0-Ca_{A}, and P_{APS,B} = CCh_{B} − 0-Ca_{B} (means ± SE, Ω indicates significant difference, P < 0.025, n = 6). B: peak P_{APS} as a fraction of P_{ref} (Ω indicates significant difference, P < 0.025, n = 6). C: effect of 10 µM STP or 1.0 µM H-1152 during CCh stimulation before the 3rd fill-empty cycle (CCh_{B} ± drug) on the subsequent normalized peak P_{APS} during passive bladder filling (P_{APS,B} ± drug = CCh_{B} ± drug − 0-Ca_{B}, Ω and * indicate values significantly greater than or less than 1.0, respectively, P < 0.05, n = 6 control, n = 3 STP and H1152).
proached but did not reach a steady-state $T_p$ value during the relatively short, 2-min, isometric period before each loading $T_p$ measurement. The greater $T_p$ during unloading at 80% $L_{\text{max}}$ in Fig. 2B compared with Fig. 1C may have been due to the level of precision with which $L_{\text{ref}}$ was determined. The smaller length steps taken in the CCh-induced $T_p$ results presented in Fig. 2 likely provided a more precise determination of $L_{\text{ref}}$, but also increased the number of contractions and time taken to identify $L_{\text{ref}}$, which may have permitted greater length adaptation of the $L$-$T_a$ curve (2, 44). Furthermore, the larger difference in normalized $T_p$ between loading and unloading following a KCl precontraction (Fig. 1C) compared with following a CCh precontraction (Fig. 2B) may also have been due to variation in $L_{\text{ref}}$ or due to a difference in their ability of KCl and CCh to generate APS at the concentrations used. The objective of these two protocols was to determine whether mouse bladder strips exhibit both KCl-induced and CCh-induced APS. Further study would be necessary to quantitatively compare the relative ability of KCl and CCh to induce APS in mouse bladder strips.

This is the first study to identify APS in mouse bladder and the first study to identify APS in whole bladders from any species. APS in rabbit DSM strips can be responsible for a substantial fraction of $T_p$ and total tension (1). Under the experimental conditions of the present study, passive pressure attributed to APS was responsible for a substantial percentage, 12–16%, of $P_{\text{ref}}$ (Fig. 6B) and was therefore a substantial fraction of wall tension during mouse bladder filling.

APS in a mouse PBOO model. Morphological studies examining PBOO mouse bladders indicate that there are significant changes in both smooth muscle and extracellular matrix that occur with obstruction. Austin et al. (3) studied a male murine model of PBOO identical to that used in the current study, except that the duration of obstruction was 4 wk compared with 2 wk. They showed that severe (decompensated) obstruction can be differentiated from mild (compensated) obstruction based on large increases in peak volumes and moderate decreases in peak pressures using KCl contractions or electrical field stimulation. These authors and others showed that severe obstruction is associated with increases in the myosin-A-to-myosin-B isoform ratio as well as increases in extracellular...
collagen (3). The progression from inflammation to fibrosis has been studied in a rat PBOO model (32), and increased expression of bladder glycogen has also been shown to correlate with the degree and time course of obstruction and may be used as an obstruction marker (15).

In the present study, PBOO bladders were categorized as compensated PBOO and decompensated based on the work of Austin et al. (3) in which the groups can be clearly defined based on volume ($V_{ref}$). $P_{APS}$ was elevated during filling in bladders subjected to 2 wk of PBOO (Fig. 9). The increase in $P_{APS}$ in the compensated PBOO bladders was likely due to elevated cross-bridge formation as more chronic changes in extracellular collagen were not likely to have occurred (3). On the other hand, observed changes in pressure and volume in the decompensated PBOO bladders may have been due to APS as well as chronic changes in the DSM myosin isoforms and extracellular collagen content (3). Austin et al. (3) compared bladders from control and sham-operated mice and did not identify a difference in bladder mass, $V_{ref}$, or $P_{ref}$; therefore, we attribute the differences in $P_{ref}$ and $P_{APS}$ in PBOO bladders compared with the control bladders, which only included one sham-operated bladder, to the obstruction and not to the surgery alone. The observed differences in the decompensated PBOO group may also have been due to the small number of decompensated bladders identified in the current study. Only two of the six PBOO bladder studies were categorized as decompensated, and although this number was too small to determine statistical significance, we felt that it was important to report data from this group. Approximately 70% of PBOO bladders exhibited severe hypertrophy 4 wk after PBOO in Austin’s model (3), and therefore we anticipate that a greater number of decompensated bladders would have been identified in the present study if the duration of PBOO was extended beyond 2 wk. It is tempting to speculate that elevated APS-dependent passive pressures exhibited by the compensated PBOO group may serve as a mechanism to make up for the diminished ability to generate active pressure (Fig. 8). These data also demonstrate the potential importance of APS as a target for therapy in human voiding dysfunction secondary to bladder outlet obstruction.

**Regulation of APS in mouse bladder.** We originally gave APS the title “adjustable passive stiffness” (47) because this tension component was measured when tissues were “at rest” (i.e., not stimulated to contract) and incubated in a Ca$^{2+}$-free solution in which Ca$^{2+}$-dependent cross bridges do not form. Passive tension is measured in “resting” muscle to distinguish it from the active tension caused by the action of contractile stimuli that elevate cellular Ca$^{2+}$ and initiate actomyosin cross-bridge cycling. However, studies subsequent to our initial observation show that APS is an actively regulated component of DSM tension (1, 37). The most surprising feature is that APS is retained when tissues are incubated in a Ca$^{2+}$-free solution. For example, all loading cycles in Figs. 1C and 2B and all filling cycles in Figs. 4A and 5A were performed in Ca$^{2+}$-free solution, and the levels of $T_a$ and filling pressure were adjustable based on the pretreatment before each loading or filling cycle. Thus, our data support the hypothesis that APS is due to actomyosin cross bridges that appear to “hold” tension for long durations, even when tissues are incubated in a Ca$^{2+}$-free solution. For example, the generation of APS in rabbit DSM can be inhibited by the ROCK inhibitors H-1152 and Y-27632 (37, 45), the actin polymerization inhibitor latrunculin-B (37), and the selective myosin II inhibitor blebbistatin (37). APS was recently identified in sheep airway smooth muscle, and in this cell type, APS is attributed at least in part to dense body cables (52). The present study indicates that APS generation in both mouse bladder strips and whole bladder is due, at least in part, to inhibition of a serine/threonine protein kinase because STP reduced APS by ~40%. APS was likewise inhibited by ~40% by 1 μM H-1152, a concentration that inhibits ROCK by 97% (5). Thus, it is likely that APS generation is an active process involving ROCK regulation. However, because H-1152 is not entirely selective and can modestly inhibit conventional and atypical PKC isotypes by ~32% (5), it is possible that other kinases also play a role. Together, these data are consistent with a model suggesting that restoration of APS is due to an increase in myosin light chain phosphorylation resulting in activation of slowly detaching cross bridges (1, 37). Furthermore, the reduction in active pressure and corresponding increase in passive pressure demonstrated by the PBOO bladders (Fig. 8A) suggest that PBOO may lead to a repurposing of cross bridges from active pressure generation to passive pressure maintenance.

The fraction of $T_a$ abolished in rabbit DSM strips solely by incubation in deionized water (1) and the loss in passive pressure in mouse bladder due to incubation in deionized water (Fig. 7) could be due to purely mechanical viscoelastic structures and/or fluid redistribution within DSM cells, or to biochemically regulated APS. The presence of APS in mouse bladder will permit future studies of APS regulatory pathways and potential alterations of APS in disease models using knockout transgenic mice.

**Bladder activity during filling.** Mammalian bladders, including those from mice (19, 25, 29), rats (16, 27), guinea pigs (17), rabbits (41), cats (22), and humans (7), are not considered to be “at rest” during filling because they display spontaneous rhythmic contractions. Spontaneous rhythmic contraction during filling represents a nonvoiding contraction because the contractile amplitude is significantly weaker than the contraction responsible for voiding (13). For example, spontaneous rhythmic contraction of rabbit DSM is ~5–12% of peak $T_a$ induced by KCl or CCh (36). Thus, there are two distinct contractile phenomena in the bladder, spontaneous rhythmic contraction during the filling phase and voiding contraction during the
voiding phase. Furthermore, there are two regulated components of bladder wall tension during the filling phase, spontaneous rhythmic contraction and APS, and these two components appear to be integrally related for a number of reasons. First, APS in rabbit DSM can be established by CCh-induced or PGE$_2$-induced rhythmic contraction (1). Second, spontaneous rhythmic contraction (36) and APS (37, 45) can be regulated by inhibition of ROCK activity. Finally, evidence supports the hypothesis that actomyosin cross bridges, which are responsible for spontaneous rhythmic contraction, are also responsible for APS (1, 37).

Potential physiological significance of APS. The presence of APS in mouse bladder was revealed through multiple filling cycles (Fig. 4) similar to cystometry used to evaluate overactive bladder. Whether human bladders exhibit APS remains to be determined; however, examination of earlier studies involving repeated cystometry provides evidence for the presence of APS in human bladders. For example, in one of the earliest human cystometric studies, Simeone and Lampson (43) showed a substantially more compliant P-V relationship during cystometry following treatment of colon impaction and an additional increase in compliance 24 h later. Ockrim et al. (34) showed that DSM overactivity varies during repeated cystometry on patients with urgency, with the greatest correlation between DSM overactivity and urgency occurring during a third filling. Furthermore, some patients adapted to repeated filling while others did not, and those that did not adapt showed stronger urgency (34). We anticipate that human bladders will be shown to exhibit APS and speculate that APS may contribute to the variability in repeated cystometry in patients with urgency. This is the first study to examine APS in an animal model of voiding dysfunction. PBOO is an established model of detrusor overactivity (35, 38) and the identification of elevated APS in bladders from PBOO mice (Fig. 9) indicates that APS may be relevant in diseases affecting bladder mechanics. Tension sensors “in-series” with afferent nerves are activated by increased bladder volume and bladder wall contraction (18), and elevated spontaneous rhythmic contraction is associated with increased urgency (10, 22). Any change in bladder wall compliance due to spontaneous rhythmic contraction and/or APS must affect these tension sensors to some degree. If a molecular structure responsible for APS (or spontaneous rhythmic contraction) is in series with a tension sensor, then both elements would support the same tension, just as all links in a single long chain bear the same load. Therefore, when stretched to a particular muscle length, the tension in both the APS (or spontaneous rhythmic contraction) element and the sensor would be greater if the APS element was stiffer (or if the structure responsible for spontaneous rhythmic contraction contracted). If the sensor is calibrated to trigger at a specific tension level, then it would reach this threshold at a shorter muscle length (smaller volume) when the stiffness of the in-series APS was elevated (or if the spontaneous rhythmic contraction element contracted), and this would likely lead to premature urgency. Thus, a dysfunction in the structures responsible for $T_p$ during the filling phase, including those responsible for providing and regulating APS, could directly contribute to OAB. A better understanding of DSM activity during filling, including the regulation of APS, is necessary and may provide important insight into both the molecular mechanisms that contribute to OAB and into the cystometric evaluation of patients with OAB.

ACKNOWLEDGMENTS

Expert technical assistance was provided by Amy S. Miner. Bladders from PBOO mice were kindly provided by Dr. Samuel Chacko and Dr. Stephen Zderic at the George M. O’Brien Urology Research Center at the University of Pennsylvania. We thank Dr. Chacko and Dr. Zderic for valuable assistance with this model and the generous contribution to the research. We also thank Stephan Butler for technical assistance with the PBOO bladders.

GRANTS

This study was supported by a grant from the Edwin Beer Research Program in Urology and Urology Related Fields from the New York Academy of Medicine (to J. E. Speich). Some bladders used for these studies were from mice used for vascular smooth muscle studies supported by National Heart, Lung, and Blood Institute Grant RO1-HL61320 (to P. H. Ratz). S. Henderson was supported through the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) Short-Term Education Program for Underrepresented Groups (STEP-UP) for undergraduates. George M. O’Brien Urology Research Center at the University of Pennsylvania was supported by NIDDK Grant P50-DK052620 (to Dr. S. K. Chacko).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


