Transient contractions of urinary bladder smooth muscle are drivers of afferent nerve activity during filling

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Activation of afferent nerves during urinary bladder (UB) filling conveys the sensation of UB fullness to the central nervous system (CNS). Although this sensory outflow is presumed to reflect graded increases in pressure associated with filling, UBs also exhibit nonvoiding, transient contractions (TCs) that cause small, rapid increases in intravesical pressure. Here, using an ex vivo mouse bladder preparation, we explored the relative contributions of filling pressure and TC-induced pressure transients to sensory nerve stimulation. Continuous UB filling caused an increase in afferent nerve activity composed of a graded increase in baseline activity and activity associated with increases in intravesical pressure produced by TCs. For each ∼4-mmHg pressure increase, filling pressure increased baseline afferent activity by ∼60 action potentials per second. In contrast, a similar pressure elevation induced by a TC evoked an ∼10-fold greater increase in afferent activity. Filling pressure did not affect TC frequency but did increase the TC rate of rise, reflecting a change in the length-tension relationship of detrusor smooth muscle. The frequency of afferent bursts depended on the TC rate of rise and peaked before maximum pressure. Inhibition of small- and large-conductance Ca^{2+}-activated K^+ (SK and BK) channels increased TC amplitude and afferent nerve activity. After inhibiting detrusor muscle contractility, simulating the waveform of a TC by gently compressing the bladder evoked similar increases in afferent activity. Notably, afferent activity elicited by simulated TCs was augmented by SK channel inhibition. Our results show that afferent nerve activity evoked by TCs represents the majority of afferent outflow conveyed to the CNS during UB filling and suggest that the maximum TC rate of rise corresponds to an optimal length-tension relationship for efficient UB contraction. Furthermore, our findings implicate SK channels in controlling the gain of sensory outflow independent of UB contractility.

INTRODUCTION

The urinary bladder (UB) has two key functions: to store and void urine. Voiding occurs through the coordinated contraction of detrusor smooth muscle cells in the bladder wall. Gradual increases in bladder pressure associated with filling activate afferent sensory nerves, a linkage that has been suggested to communicate a sense of fullness to the central nervous system (CNS; de Groat and Yoshimura, 2009). Although aberrant sensory feedback has been implicated in multiple bladder pathologies (Araki et al., 2008), the mechanisms involved in the sensation of bladder fullness are still unclear. It is also unknown whether detrusor smooth muscle is integrally involved in communicating a sense of fullness or sensing pressure increases during bladder filling.

In addition to contractions that void urine, detrusor smooth muscle in normal bladders from a variety of species (including humans) exhibits nonvoiding contractions in vivo during filling (Robertson, 1999; Streng et al., 2006; Zvara et al., 2010; Biallosterski et al., 2011). Nonvoiding contractions are also more likely to occur and are more frequent in UB pathologies (Bristow and Neal, 1996; Brading, 1997; Fowler et al., 2008; Gillespie et al., 2012; Li et al., 2013). Similar transient contractions (TCs) are also present in ex vivo preparations, where they have been termed "micromotions" or "spontaneous phasic contractions," and appear to reflect local smooth muscle contractions in the bladder wall (Drake et al., 2003; Gillespie, 2004; Parsons et al., 2012; Vahabi and Drake, 2015). Previous studies also observed afferent nerve activity accompanying these contractions of the bladder wall in ex vivo and in vivo murine preparations (Iijima et al., 2009; McCarthy et al., 2009; Yu and de Groat, 2010, 2013; Zvara et al., 2010; Daly et al., 2014). These observations suggest that TCs of the detrusor smooth muscle might have a role in encoding information on the state of bladder fullness. Although previous studies have suggested an association between TCs and afferent activity (Satchell and Vaughan, 1989; Yu and de Groat, 2008; Iijima et al., 2009; Kanai and Andersson, 2010), a systematic investigation of the role of TCs in controlling afferent activity is lacking.

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Abbreviations used in this paper: CNS, central nervous system; TC, transient contraction; TTX, tetrodotoxin; UB, urinary bladder; VDCC, voltage-dependent Ca^{2+} channel.

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TCs are caused by Ca^{2+} influx through L-type voltage-dependent Ca^{2+} channels (VDCCs) during detrusor smooth muscle action potentials. The upstroke of these action potentials is caused by opening of VDCCs, and repolarization phases are mediated by voltage-dependent K^+ (K_V) channels, large-conductance Ca^{2+}-activated K^+ (BK) channels, and small-conductance Ca^{2+}-activated K^+ (SK) channels (Heppner et al., 1997, 2005; Herrera et al., 2000; Hashitani and Brading, 2003a,b; Thorneloe and Nelson, 2003; Young et al., 2008; Nausch et al., 2010). BK and SK channels are of particular interest because knockout of either channel results in an overactive bladder phenotype, characterized by detrusor hyperactivity and increased micturition frequency (Herrera et al., 2003; Meredith et al., 2004; Thorneloe et al., 2005). Blocking BK or SK channels also increases TCs in detrusor smooth muscle strips, indicative of an increase in detrusor smooth muscle excitability (Herrera et al., 2000; Buckner et al., 2002; Hashitani and Brading, 2003b). Interestingly, recent findings indicate that SK channels are also present in a subset of platelet-derived growth factor receptor-α (PDGFRα)–positive, interstitial cells within the bladder wall (Lee et al., 2013). Although the function and nature of bladder interstitial cells are unclear, these cells may receive and transduce neural signals to and from detrusor smooth muscle via their close association with nerve varicosities within the bladder wall (Koh et al., 2012; McCloskey, 2013). Thus, it remains unknown whether BK or SK channels play a role in the sensation of bladder fullness or the transduction of bladder fullness into afferent nerve activity or whether they simply serve to regulate detrusor smooth muscle excitability.

Here, we explore the relationship between TCs and afferent nerve activity, testing the hypothesis that TCs have an outsized influence on sensory outflow. We also explore the roles of BK and SK channels in the transduction of pressure changes into sensory nerve outflow. We demonstrate that the waveform of a TC is uniquely suited to elicit sensory nerve activity, despite its relatively small amplitude; furthermore, this waveform is determined by VDCCs and BK channels in detrusor smooth muscle. Moreover, the rate of rise, but not the frequency, of TCs increases with filling pressure, which leads to an increased contribution of TCs to afferent nerve activity. Somewhat surprisingly, SK channels act independently from detrusor smooth muscle activity to regulate the transduction of mechanical deformation into afferent activity. Our findings are consistent with the idea that TC-induced bursts of afferent nerve activity play an important role in conveying a sense of bladder fullness and regulating micturition frequency. Furthermore, we suggest that SK channels, perhaps in interstitial cells within the bladder wall, regulate bladder sensory outflow by limiting the gain during mechanotransduction of TCs to afferent activity and not necessarily by altering detrusor contractility. These findings underscore the potential utility of therapeutic agents that decrease detrusor smooth muscle contractility or its communication to afferent nerves in the treatment of lower urinary tract symptoms.

MATERIALS AND METHODS

Ex vivo bladder preparation

Male C57BL/6 mice (3–4 mo of age) were euthanized by intraperitoneal injection of 150 mg/kg sodium pentobarbital followed by decapitation, in accordance with protocols approved by the Institute of Animal Care and Use Committee of the University of Vermont. The UB, with ureters, urethra, major pelvic ganglia, and pelvic nerves attached, was removed and placed in ice-cold HEPES-buffered physiological saline solution (HB-PSS) consisting of 134 mM NaCl, 6 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 10 mM HEPES, and 7 mM glucose, pH 7.4. The ureters were tied adjacent to the bladder wall with 4-0 sutures, and the pelvic nerves were exposed and cleaned of connective tissue before placing the bladder preparation in the recording chamber. In the recording chamber, the HB-PSS was replaced with bicarbonate-buffered physiological saline solution (PSS) consisting of 118.5 mM NaCl, 4.6 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgCl_2$, 2 mM $CaCl_2$, 24 mM $NaHCO_3$, and 7 mM glucose; the pH of the solution was maintained at 7.4 by bubbling with 20% O_2/5% CO_2. All experiments were performed at 37°C. The urethra was cannulated and attached to a syringe pump, allowing for continuous infusion of PSS into the bladder. UBs were continuously filled with PSS at 1.8 ml/h to a pressure of 25 mmHg, at which point filling was stopped and the bladder was allowed to empty. The maximum bladder pressure chosen was based on cystometric recordings from normal C57BL/6 mice, which indicated that voiding threshold was reached at ∼11 mmHg and peak contraction pressures occurred at ∼26 mmHg (Herrera et al., 2003). Bladder pressure was measured using a pressure transducer placed in the infusion line next to the bladder and connected to a Living Systems Pressure Servo Controller, Model PS-200 (Living Systems Instrumentation). One of the pelvic nerves distal to the pelvic ganglia was attached to a suction electrode for electrophysiological recordings. All drugs were added directly to the recirculating bath, as well as intravesically (where noted).

Electrophysiology

One of the pelvic nerves was attached to a fire-polished glass tip (tip opening, ∼100 µm) of a suction electrode to detect action potentials. Action potentials from the pelvic nerve were collected using a NeuroLog headstage (NL100AKS, Digitimer), amplified with an AC preamplifier (NL104; Digitimer), and band-pass filtered at 200–4,000 Hz (NL125/NL126; Digitimer) to remove noise. Data were collected and stored using a Power 401 analogue to digital interface and Spike 2 software (Cambridge Electronic Design). Pressure was acquired at a rate of 100 Hz, and afferent activity was acquired at a rate of 25,000 Hz. Action potential occurrence was determined by setting the detection threshold to twice the root mean square of the recorded signal in the absence of action potentials. Action potential frequency was then calculated from action potential occurrence (see Fig. 1 A) using Spike 2 software. For comparisons with other published studies (Yeh et al., 2010; Zvara et al., 2010; Daly et al., 2014; Nocchi et al., 2014), afferent activity was defined as the frequency of action potentials per second (Hz). The mean frequency at each event was calculated by counting the number of events occurring during the 0.1 s immediately preceding the event. These data (pressure and action potential frequency) were then exported for offline analysis.

Afferent activity analysis

Afferent nerve activity during bladder filling is composed of two components: baseline afferent activity and superimposed, rapid increases in afferent activity evoked by TCs. Each portion of afferent activity was analyzed independently.

Afferent activity, measured as intravesical pressure increased during bladder filling, was first averaged in 1-s bins. Baseline activity was determined for every 2-mmHg change in intravesical pressure. Because the afferent activity associated with superimposed TCs creates periodic spikes above baseline, it was necessary to estimate baseline activity from raw recordings using an analysis procedure that essentially subtracted TC-associated afferent activity. To this end, we identified the lowest afferent nerve activity occurring within 10 s before and after the last point each 2-mmHg increment in pressure was reached. These two values (which necessarily exclude the higher TC-associated afferent activity) were averaged, and this mean value was considered the baseline afferent activity for that particular pressure.

TCs and concomitant afferent activity were measured and analyzed using LabChart 7 Pro software (AD Instruments). Only TCs occurring at baseline intravesical pressures between 0 and 12 mmHg were measured because the rapid change in baseline pressure that occurred above 12 mmHg prevented accurate peak analysis. Furthermore, pressures >12 mmHg are only reached during bladder emptying, not bladder filling (Herrera et al., 2003). The start time, amplitude, duration, leading slope, and trailing slope were recorded for both the TC and the concomitant increase in afferent activity. TC duration was measured as the elapsed time above 50% maximum amplitude. TC leading slope and trailing slope were calculated by linear regression of all points between 20 and 80% maximum amplitude on either side of the peak. TC mean frequency was measured by counting the number of TCs occurring during a fixed period (between 1 and 4 min) within the intravesical pressure ranges indicated.

Drugs

Diltiazem and nifedipine were obtained from Sigma-Aldrich, paxilline was obtained from Enzo Life Sciences, tetrodotoxin (TTX) was obtained from Tocris Bioscience, and apamin was obtained from Peptides International.

Statistical analysis

For comparisons of two samples of equal variance, statistical significance between groups was assessed using two-tailed, paired or unpaired Student's t tests ($\alpha = 0.05$). For multiple comparisons, an ordinary or repeated-measures one-way ANOVA was used followed by Bonferroni's post hoc analysis to compare individual means. Calculations were performed using Excel (Microsoft Corporation) or Prism (GraphPad Software). Unless otherwise indicated, "N" represents the number of animals in each group, whereas "n" represents samplings taken from within these groups.

RESULTS

Afferent nerve activity and TCs during bladder filling

To investigate what drives afferent outflow during filling and emptying of the UB, we used an ex vivo bladder preparation with intact urethra, major pelvic ganglia, and pelvic nerves. This preparation allows for simultaneous recording of afferent nerve activity and bladder pressure during bladder filling (Fig. 1 A). Afferent activity was low in the empty bladder, but steadily increased during bladder filling as bladder volume and intravesical pressure increased. This steady increase in afferent

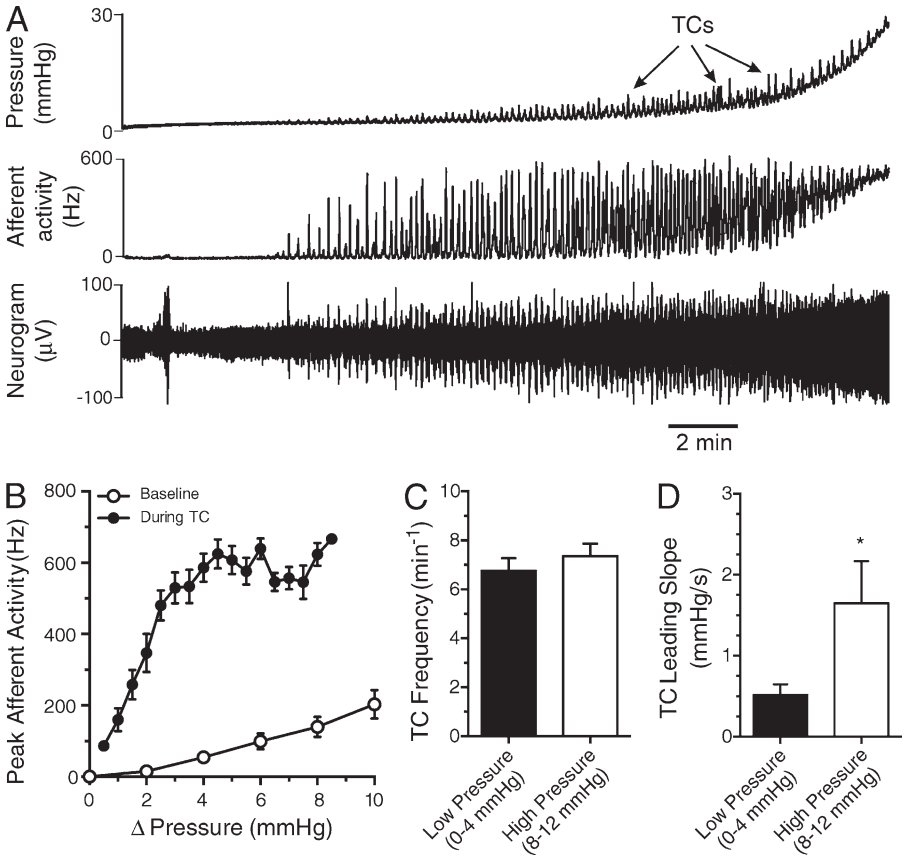

Figure 1. Pressure increases during TCs stimulate afferent nerve activity. (A) Pressure and afferent nerve activity were recorded simultaneously from an ex vivo bladder preparation during filling. Pressure response with small TCs (top trace) and afferent nerve activity (mean frequency, middle trace; raw data, bottom trace) during bladder filling. (B) Baseline afferent activity during bladder filling and peak afferent activity during TCs. (C and D) Mean TC frequency (C) and leading slope (D) at low (0–4 mmHg) and high (8–12 mmHg) pressures. *, $P < 0.05$ versus control; paired Student's t test ($N = 7$). Error bars represent ±SEM.

activity, termed baseline afferent activity, showed a nearly linear increase with pressure (Fig. 1 B). Most bladders (~88%) also exhibited TCs during bladder filling cycles. TCs varied in amplitude (0.2–4.0 mmHg; n = 440 from 10 animals) and duration (2–4.6 s; n = 440 from 10 animals) at filling pressures between 0 and 12 mmHg and coincided with transient bursts of afferent nerve activity. Thus, afferent nerve activity was a combination of baseline afferent activity, which increased steadily with increasing intravesical pressure, and transient afferent bursts, which were coincident with TCs (Fig. 1 A). Although both baseline pressure and TCs increased afferent nerve activity, the comparatively rapid change in pressure induced by TCs evoked a much larger increase in afferent output (Fig. 1, A and B). For example, an elevation of baseline pressure by 4 mmHg increased afferent activity by roughly 60 Hz (Fig. 1 B). By comparison, an equivalent 4-mmHg increase in intravesical pressure associated with a TC evoked a much larger (~600 Hz) increase in afferent activity (Fig. 1 B), a value well within the resolution of the recording system (~1,000 Hz). Afferent nerve activity plateaued at TC amplitudes >4 mmHg. These findings indicate that for an equal change in intravesical pressure, TCs cause a disproportionally larger increase in afferent activity compared with increases in baseline pressure and thus are capable of driving the majority of sensory nerve outflow from the bladder (Fig. 1 B). Notably, whereas pressure had no effect on TC frequency (Fig. 1 C), the rate of rise (TC leading slope) significantly increased during bladder filling (Fig. 1 D), suggesting a mechanism by which TCs could communicate bladder fullness.

Peak afferent nerve activity occurs during the rising phase of the TC and is dependent on the rate of rise

To further understand the relationship between TCs and afferent bursts, we examined single TCs and associated nerve activity. Fig. 2 A illustrates two TCs with similar amplitudes, but different rates of rise, and the associated bursts of afferent nerve activity at a low filling pressure. A closer examination revealed that a burst of nerve activity began as soon as the rising phase of the TC could be detected and reached a maximum ~1 s before the peak of the TC. The burst of afferent activity began just after the leading slope of the TC began to increase and decreased as soon as the slope began to fall (Fig. 2 B). The TC leading slope was positively correlated with the frequency of the accompanying peak afferent activity, indicating that TCs with faster leading slopes evoked afferent bursts with a greater peak frequency, saturating at a TC leading slope of ~3 mmHg/s (Fig. 2 C). Thus, the rate of pressure rise during a TC is a major determinant of afferent nerve activity.

TCs are not dependent on sensory nerve activity

It has been suggested that TCs are triggered by sensory nerves acting through a local circuit in the bladder wall (Lagou et al., 2004). To test this possibility, we measured TCs before and after application of the voltage-dependent Na^+ channel blocker TTX. TTX would not be expected to directly affect smooth muscle excitability because the upstroke of the UB action potential depends on activation of VDCCs and not Na^+ channels (Heppner et al., 1997). 1 µM TTX rapidly eliminated afferent nerve activity but had no effect on TCs (Fig. 3 A). Subsequent analyses confirmed that both TC leading

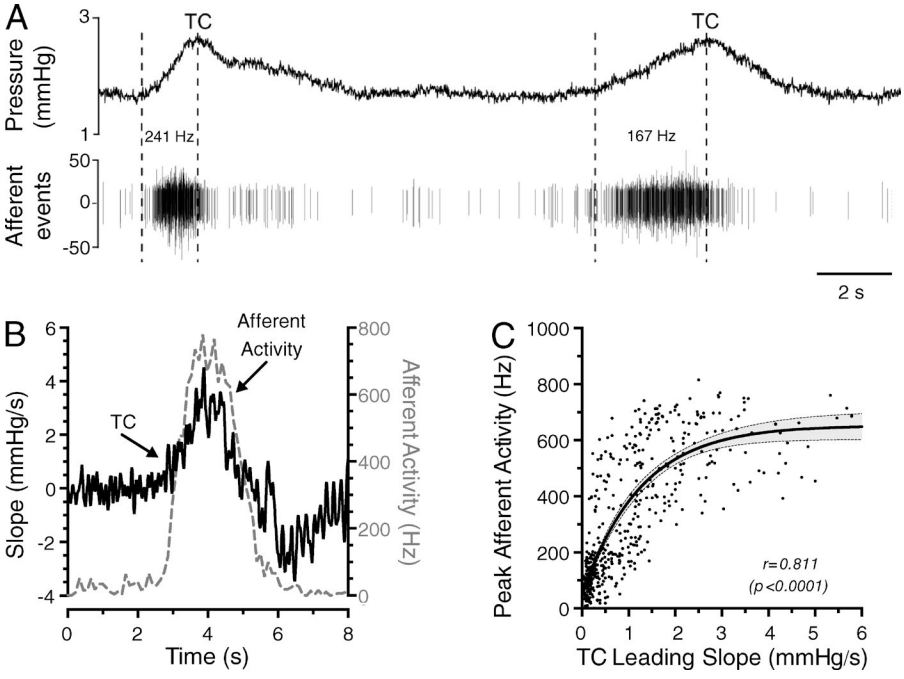

Figure 2. Afferent activity occurs primarily during the rising phase of the TC and is correlated with the TC leading slope. (A) Pressure trace (top) and afferent events (bottom) of two TCs recorded at low bladder pressure. Each vertical line (bottom) represents one action potential. The TC with the faster leading slope (left) is associated with a higher frequency of action potentials, as indicated between the vertical dashed lines. (B) Plot showing the slope of a TC (solid line) and associated afferent nerve activity (dashed line). Note that TC slope begins to increase before afferent activity and that afferent activity rapidly decreases once TC slope ceases to increase. (C) Scatter plot showing the correlation between TC leading slope and afferent nerve activity. Solid line shows a nonlinear fit of all data points. Dashed lines and shaded area indicate 95% confidence interval of a nonlinear fit (N = 8 animals; n = 485 data points).

slope (Fig. 3 B) and pressure generation (Fig. 3 C) were unaffected by TTX, whereas afferent activity (Fig. 3 D) was virtually eliminated. Collectively, these results suggest that the mechanism responsible for generating TCs is not dependent on a TTX-sensitive local nerve circuit in the detrusor wall.

BK and SK channels regulate TC leading slope and peak afferent activity

Inhibition of BK and SK channels increases detrusor smooth muscle excitability and leads to bladder overactivity (see Introduction). We therefore examined the effects of BK and SK channel inhibition on TCs and afferent nerve activity. Intravesical pressure and afferent nerve activity were recorded in the absence or presence of the BK channel inhibitor paxilline (1 μM; Fig. 4 A) or the SK channel inhibitor apamin (300 nM; Fig. 4 B). Blocking either SK or BK channels did not affect TC frequency (Fig. 4, C and F) but did affect TC rate of rise and associated afferent nerve activity. Interestingly, blocking BK channels resulted in a substantial increase in TC rate of rise (Fig. 4 D) but only a small increase in afferent activity (Fig. 4 E). In comparison, a comparatively small increase in TC leading slope caused by blocking SK channels (Fig. 4 G) resulted in a large (approximately twofold) increase in peak afferent nerve activity (Fig. 4 H). These results indicate that both BK and SK channel inhibition affect the rate of rise of TCs, but the resulting increase in afferent nerve activity is much greater with SK than BK channel block.

Baseline afferent nerve activity does not depend on L-type VDCCs, BK channels, or SK channels

As noted above, inhibition of BK or SK channels increased TC leading slope and bursts of afferent activity, respectively (Fig. 4). However, whether this action reflects effects on BK and SK channels in smooth muscle, nerve fibers, or other cell types is unclear. To further explore this, we first compared baseline afferent nerve activity in the presence of the BK channel blocker paxilline, the SK channel blocker apamin, or the VDCC blocker nifedipine (1 μM), the latter of which prevents detrusor smooth muscle action potentials and TCs (Heppner et al., 1997; Herrera et al., 2000; Buckner et al., 2002; Hashitani and Brading, 2003b). We found that nifedipine eliminated TCs, as expected; notably, it also abrogated associated bursts of afferent nerve activity (Fig. 5 A). However, VDCC inhibition did not significantly change baseline afferent nerve activity (Fig. 5 B). Likewise, inhibition of BK (1 μM paxilline) or SK (300 nM apamin) channels had no effect on baseline afferent activity compared with controls or nifedipine treatment

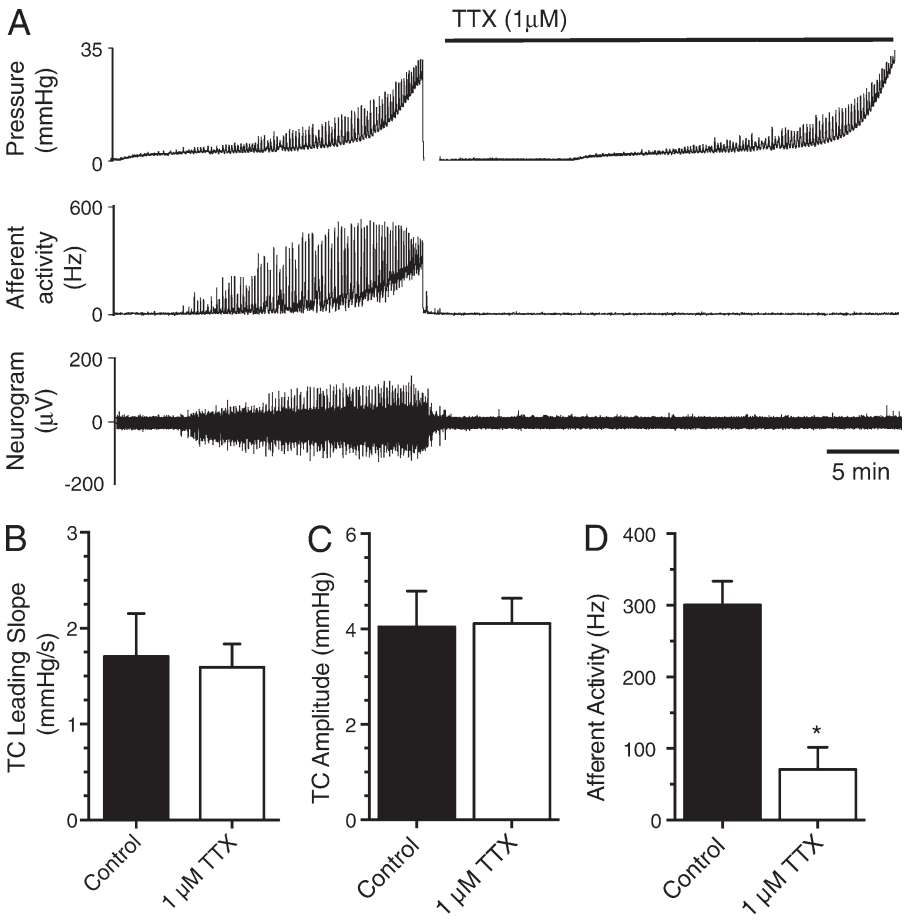

Figure 3. The voltage-dependent Na^+ channel blocker TTX eliminates afferent nerve activity but does not affect TCs. (A) Continuous recording of bladder pressure (top trace) and afferent activity (bottom trace) during sequential bladder fills, before (left) and after (right) the addition of 1 μM TTX. (B–D) Summary bar graphs showing TC leading slope (B), TC amplitude (C), and afferent activity (D) in the absence (control) and presence of TTX. *, $P < 0.05$ versus control; paired Student's t test ($N = 3$). Error bars represent ±SEM.

(Fig. 5 C), indicating that BK and SK channels do not directly regulate sensory fiber action potentials in the bladder at steady filling pressures.

TCs can be mimicked by application of a compressing force

Given that VDCC inhibition eliminated TCs completely, the experimental approach described in the previous sections was unable to distinguish whether bursts of afferent activity elicited by TCs were caused by mechanical deformation of the bladder or smooth muscle contraction per se. To further explore these relationships, we first tested whether simulated TCs, generated by gentle, transient compression of the bladder with plastic-covered forceps, was capable of generating bursts of afferent activity in the presence or absence of "natural" TCs. With the bladder filled to ~5 mmHg, very light compression from the forceps generated rapid increases in pressure and concomitant bursts of afferent nerve activity (Fig. 6, A and B), producing a TC waveform similar to that of naturally occurring TCs at the same filling pressure. As with natural TCs, the increase in leading slope coincided with the increase in afferent activity, which rapidly decreased as the leading slope fell (Fig. 6 C). Simulated TCs with the same amplitude as naturally occurring TCs (Fig. 6 D) showed a slightly increased leading slope (Fig. 6 E), likely simply reflecting inherent difficulties in simultaneously mimicking both amplitude and leading slope using a manual approach. Notably, this increase in slope resulted in a proportional increase in peak afferent activity (Fig. 6, F and G), indicating that the relationship between simulated TC leading slope and peak afferent activity was unchanged compared with that of natural TCs. Gently touching the forceps to the bladder wall (without compression) failed to evoke

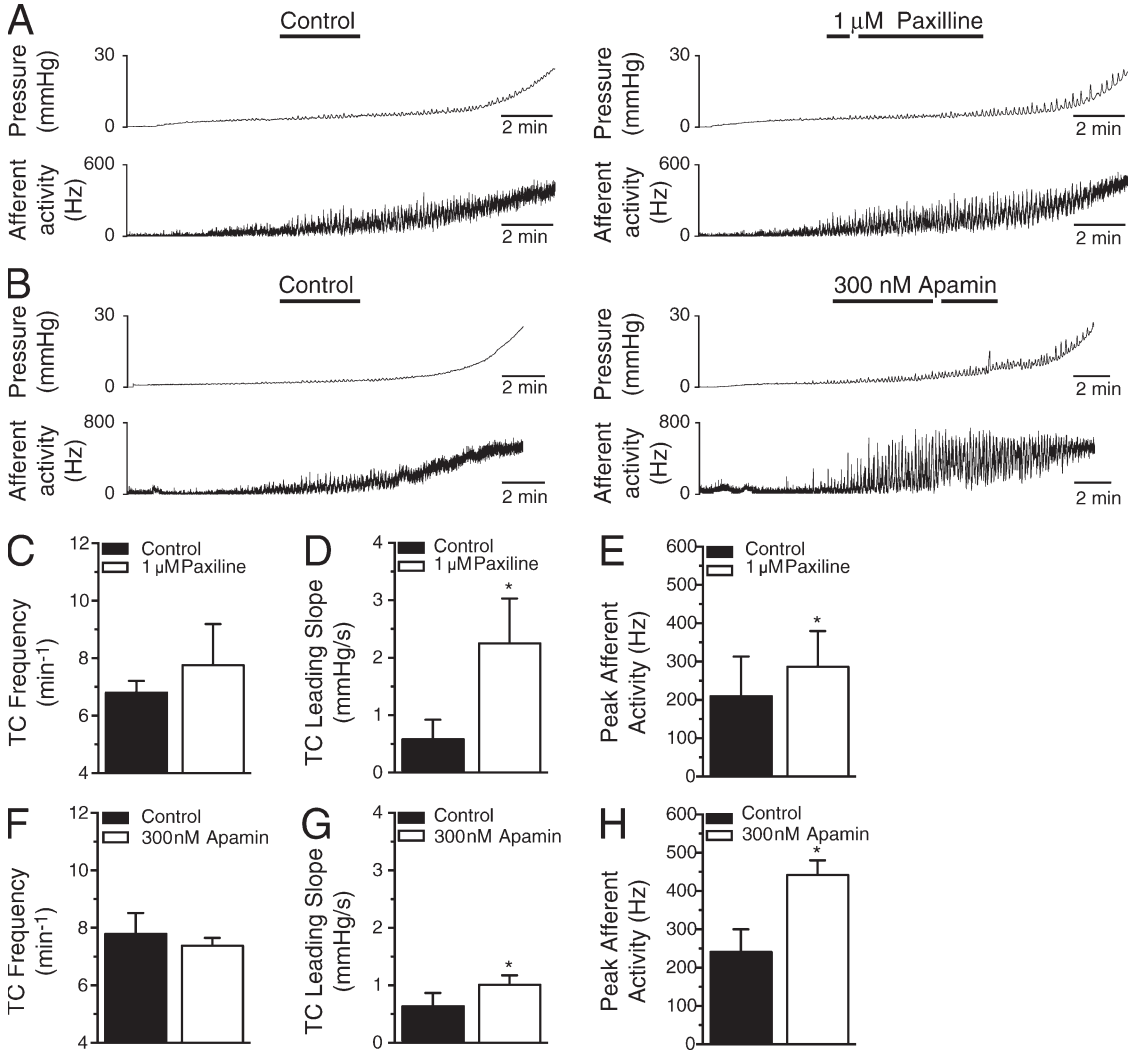

Figure 4. Blockers of BK and SK channels increase TC leading slope and afferent nerve activity. (A and B) Representative traces of pressure and afferent activity in the absence or presence of the BK channel inhibitor paxilline (1 μM; A) or the SK channel inhibitor apamin (300 nM; B). (C–E) Bar graphs illustrating the effects of 1 μM paxilline on TC frequency (C), TC rate of rise (D), and peak afferent activity (E). (F–H) Bar graphs illustrating the effects of 300 nM apamin on TC frequency (F), TC rate of rise (G), and peak afferent activity (H). *, $P < 0.05$ versus control; paired Student's *t* test (N = 5–8). Error bars represent ±SEM.

afferent bursts, indicating that the afferent bursts were not caused by contact of the external bladder wall with the forceps. These findings suggest that the deformation of the bladder produced by compression is able to substitute for the mechanical force of smooth muscle contraction. If so, the prediction is that inhibiting contraction by blocking VDCCs would have no effect on simulated TCs or evoked afferent activity. To test this, we incubated bladders with the L-type VDCC inhibitor diltiazem (50 μM), which eliminated natural TCs, before applying a compressive force. As predicted, simulated TCs generated in the presence of diltiazem still evoked bursts of afferent activity that were similar to naturally occurring TCs (Fig. 6, D–G). These results suggest that experimental bladder compression acts as a stand-in for smooth muscle contraction and imply a direct link between rapid detrusor smooth muscle contraction and afferent nerve activity.

Inhibition of SK channels, but not BK channels, increases peak afferent activity elicited by simulated TCs

To determine whether SK and BK channels modulate the transduction of mechanical force into afferent nerve activity, we generated simulated TCs in the absence or presence of paxilline (1 μM), apamin (300 nM), or the SK/IK channel opener NS-309 (10 μM). To inhibit all naturally occurring TCs, we also included the L-type VDCC inhibitor nifedipine (1 μM) in the bath. A comparison of simulated TCs of similar amplitude and rate of rise (Fig. 7, A and B) showed that SK channel inhibition significantly increased peak afferent activity (Fig. 7 C) and evoked more afferent nerve activity per given rate of rise than observed in controls (Fig. 7 D). Activation of SK channels had the opposite effect, reducing the afferent nerve activity per given rate of rise. This effect was also reversed by subsequent addition of apamin, indicating that the effects of NS-309 are mediated by SK channel activation.

In contrast to inhibition of SK channels, BK channel inhibition did not change peak afferent nerve activity or afferent activity per given rate of rise when smooth muscle activity was prevented (i.e., in the presence of nifedipine). This suggests that SK channels enhance the gain of the sensory nerve response to TCs, independent of smooth muscle. It also suggests that BK channels enhance afferent activity by increasing smooth muscle excitability.

DISCUSSION

Using an ex vivo bladder preparation, we examined afferent activity during bladder filling and assessed the

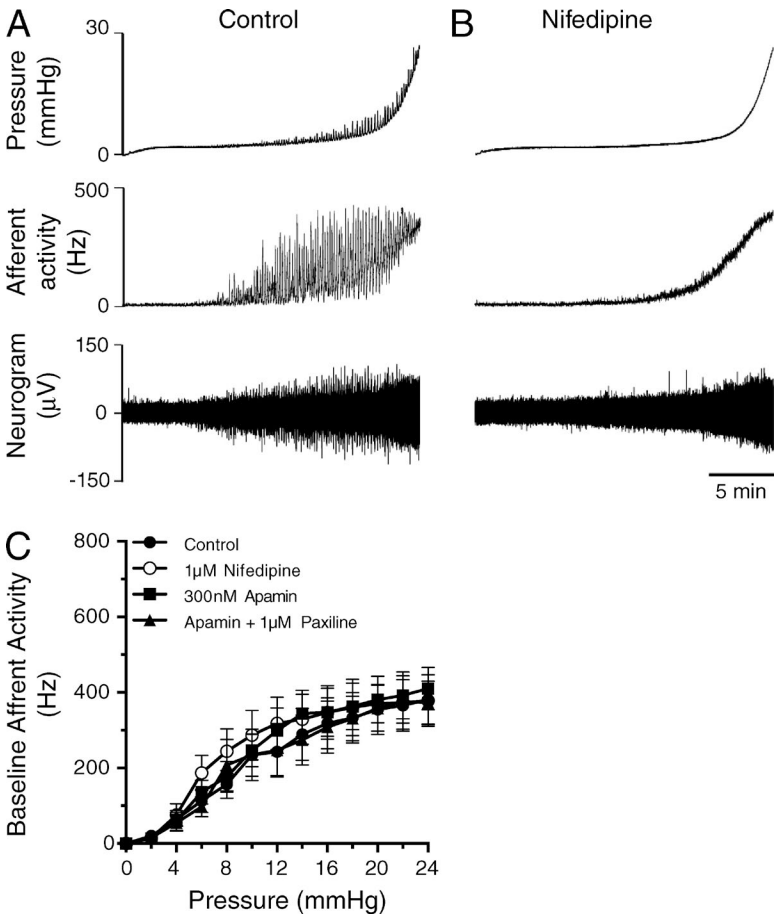

Figure 5. Baseline afferent nerve activity does not depend on L-type VDCCs, BK channels, or SK channels. (A and B) Pressure response with small TCs (top) and afferent nerve activity (mean frequency, middle; raw data, bottom) during bladder filling in the absence (A) and presence (B) of the VDCC inhibitor nifedipine (1 μM). (C) Relationship between bladder pressure and baseline afferent nerve activity in the absence (control) and presence of 1 μM nifedipine, 300 nM apamin, or 1 μM paxilline (N = 4). Error bars represent ±SEM.

specific contribution of TCs to total afferent activity. We found that baseline afferent activity increased steadily as the bladder filled and pressure increased. Superimposed on the steady increase in afferent activity were afferent bursts, similar to those described previously (Iggo, 1955; Yu and de Groat, 2008; Iijima et al., 2009; McCarthy et al., 2009) that were coincident with TCs.

For a given increase in bladder pressure, TCs generated significantly greater afferent activity compared with baseline afferent activity. For example, at subthreshold bladder pressures for micturition (<10 mmHg), a 4-mmHg increase in intravesical pressure during a TC evoked ~10-fold greater afferent activity than that induced by baseline afferent activity associated with the same increase in pressure. TC-associated afferent bursts reached a maximum before the peak of the TC and were related to the TC leading slope. This was somewhat surprising because, a priori, the largest amount of bladder wall stretch (i.e., the point of maximum pressure during the peak of the TC) might have been expected to generate the greatest amount of nerve activity. Detrusor smooth muscle contraction is clearly required for the TC-dependent sensory information generated during bladder filling, as indicated by the fact that inhibition of VDCCs eliminated both TCs and associated afferent activity. This communication to afferent nerves appears to be attributable to the mechanical forces produced by contraction/bladder wall deformation in and

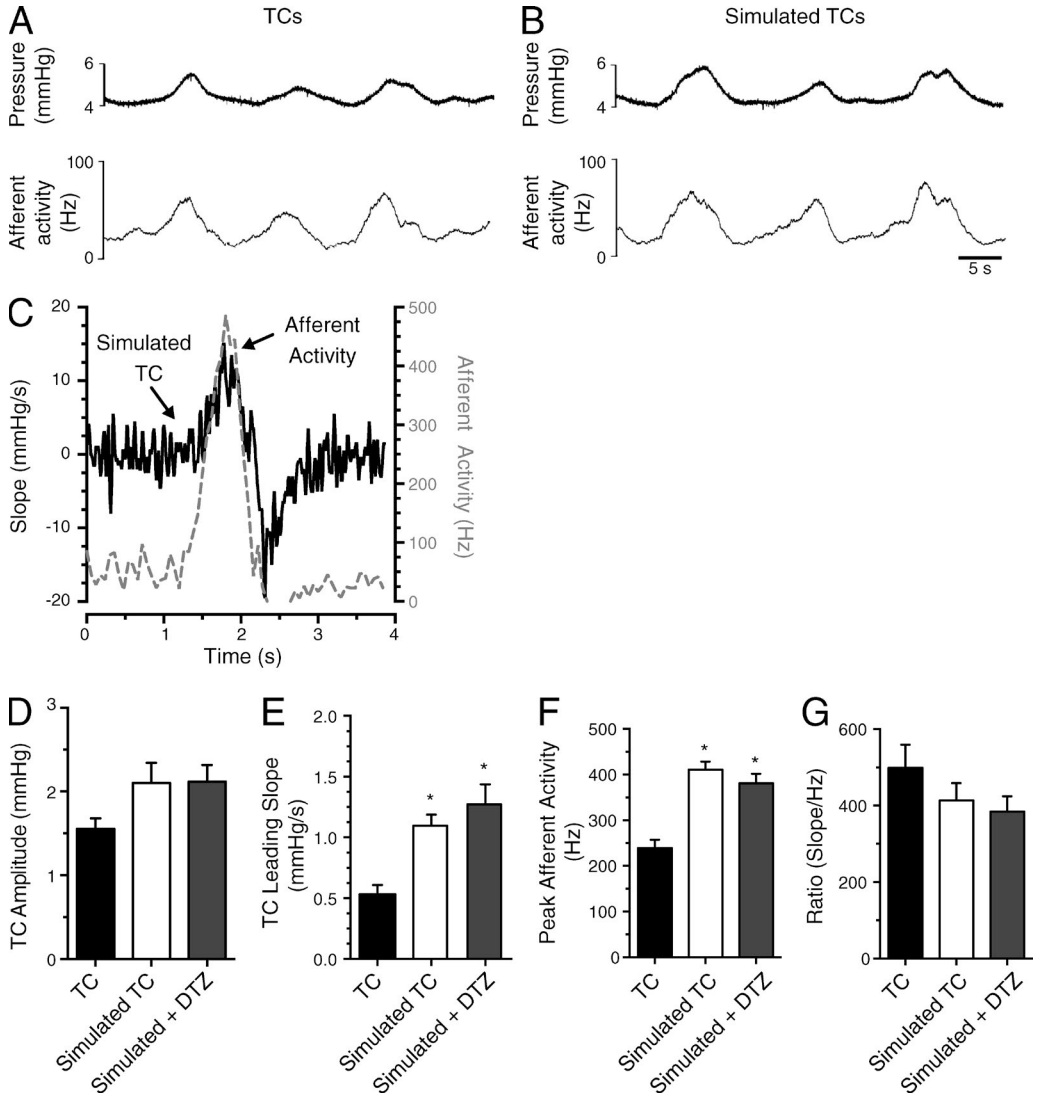

Figure 6. Simulated TCs evoke bursts of afferent activity similar to those associated with naturally occurring TCs. (A and B) Pressure curves (top) and afferent nerve activity (bottom) showing naturally occurring TCs (A) and simulated TCs (B). (C) Temporal relationship between TC slope (solid line) and afferent nerve activity (dashed line). (D–G) Comparison of total TC amplitude (D), TC leading slope (E), peak afferent activity (F), and the ratio of TC leading slope to afferent activity (G) between naturally occurring TCs and simulated TCs. Inhibition of VDCCs with 50 μM diltiazem (DTZ) did not alter simulated TC amplitude or leading slope or afferent activity. It also had no effect on the relationship between TC leading slope and afferent activity. *, $P < 0.05$ versus control; one-way ANOVA with Bonferroni's post hoc test (N = 3–5 animals; n = 12–22 events). Error bars represent ±SEM.

of itself because simulating bladder deformation (as would occur during a TC) also generated afferent bursts similar to those generated by naturally occurring TCs. Taken together with the link between TC leading slope and peak afferent activity, this suggests that afferent nerves respond rapidly to dynamic changes in bladder wall deformation to encode bladder contractility information to the CNS. The increase in the leading slope of the TC, and hence afferent activity, with filling pressure provides a mechanism by which TCs can encode information about bladder fullness to the CNS.

Simultaneous electroencephalography recordings and cystometry experiments using a model of bladder overactivity support the concept that nonvoiding contractions encode sensory information to the micturition centers of the brain (Rickenbacher et al., 2008). Nonvoiding contractions are also found in healthy volunteers during the bladder-filling phase, suggesting that these localized phasic contractions are normal events (van Waalwijk van Doorn et al., 1992; Vaughan and Satchell, 1995; Drake et al., 2005). Notably, bursts of afferent activity can increase the reliability of information transfer across synapses compared with isolated spikes (Krahe and Gabbiani, 2004). Stimulus strength, which is a determinant of primary sensory neuron discharge frequency, is greater for TCs than for increases in baseline pressure. Thus, by analogy to synaptic communication between neurons in the CNS, burst firing associated with TCs may increase the strength and reliability of synaptic transmission in the spinal cord and exert a greater impact on the sensory processing that leads to micturition. Nonetheless, an increase in these events may contribute to bladder pathogenesis in humans and animals (Gillespie, 2004; Vahabi and Drake, 2015). Therefore, we propose that nonvoiding contractions send significant sensory information to higher centers that may influence behavior and micturition frequency.

The nature and pressure dependence of transient bladder contractions

Transient or nonvoiding contractions likely reflect an increase in excitability of a small fraction of smooth muscle cells in the bladder wall. Although these events have been referred to as spontaneous phasic contractions, this nomenclature belies the fact that these events are responsive to and shaped by external forces. Indeed, the frequency of TCs varies from bladder to bladder and increases in pathology (Coolsaet et al., 1993; Drake et al., 2003; Gillespie, 2005; Biallosterski et al., 2011). The mechanisms that determine the frequency of TCs are not known but would clearly affect sensory outflow. It is conceivable that one role of the numerous and different types of interstitial cells in the bladder is to coordinate TCs and their frequency; nonetheless, our data suggest that pressure does not affect the frequency of TCs.

Intravesical pressure did increase the rate of rise of TCs, such that the maximum impact of a TC on afferent nerve activity occurs at micturition threshold pressure (10–12 mmHg; Figs. 1 D and 2 C). This dependence on pressure could be simply explained by the length-tension relationship of the detrusor smooth muscle, given

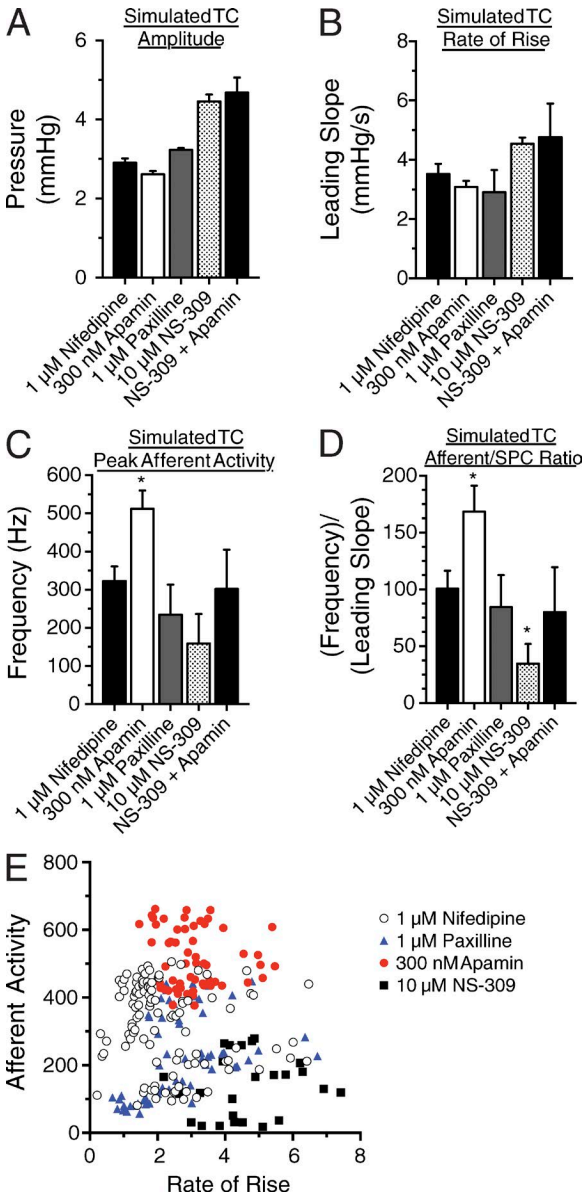

Figure 7. SK channel inhibition increases peak afferent activity elicited by simulated TCs. (A–D) Comparison of simulated TC amplitude (A), TC leading slope (B), and peak afferent activity (C) and the ratio of TC leading slope to afferent activity (D) in the presence of the SK blocker apamin (300 nM), the BK channel blocker paxilline (1 µM), the SK/IK activator NS-309 (10 µM), or NS-309 and apamin together. The Ca^{2+} channel blocker nifedipine (1 µM) was also added to block naturally occurring TCs. (E) The relationship between TC leading slope and afferent activity for all simulated TCs. *, $P < 0.05$ versus control; paired Student's *t* test (N = 3–6 animals; *n* = 10–20 events). Error bars represent ±SEM.

that the maximal contractile force is generated only after the addition of some degree of stretch or elongation (Gordon et al., 1966a,b; Cooke and Fay, 1972). If the passive tension is too little, the muscle is unable to maximally contract; if the passive tension is too high, the muscle cannot contract efficiently against the load. This is true of bladder smooth muscle as well, as the length-tension relationship during transient "cyclic contractions" of bladder smooth muscle strips remains consistent during contractions induced by multiple agonists (Speich et al., 2005). The nature of the length-tension relationship in the bladder may facilitate complete and efficient emptying of the UB, even when voiding must be delayed. Interestingly, the TC rate of rise is fastest and associated afferent activity is greatest at intravesical pressures equivalent to micturition threshold pressures recorded in vivo (Herrera et al., 2003). This implies that the properties of cyclic contractions are indicators of the overall ability of the bladder to contract, and thus the maximal rate of rise of TCs during bladder filling is indicative of the optimal length-tension relationship of the UB as a whole. Therefore, we propose that the relationship between afferent nerve activity and the rate of rise of a TC exists to encode both sensory information with regard to bladder fullness and indicate the ability of the detrusor to maximally and efficiently contract to expel urine. This dual mechanism also provides a longer window during which efficient voiding can occur, as the bursts of sensory output maximally occur over a range of pressures. Otherwise, the sensation of fullness might not be felt until the bladder was maximally full, a delay that could lead to incomplete voiding or incontinence. This represents a simple and effective mechanism by which the balance between bladder fullness and contractile capability can be transduced to the CNS to determine the appropriate time for efficient voiding.

Transduction of TCs into afferent nerve activity

The mechanism by which TCs are transduced into afferent nerve activity is still unknown. However, a potential candidate element in the transduction of TCs into afferent activity is interstitial cells, which form a network throughout the bladder and are found as distinct populations throughout the lamina propria and detrusor. In the gastrointestinal tract, interstitial cells of Cajal are traditionally associated with communication from enteric nerves to smooth muscle, serving as pacemakers and mediators of neurotransmission (Sanders, 1996). However, no evidence exists supporting a similar relationship between bladder interstitial cells and detrusor smooth muscle (McCloskey, 2013), and thus the function of bladder interstitial cells remains obscure. A recent study found that subpopulations of these cells respond to electrical field stimulation with a transient increase in Ca^{2+}, indicating that these cells are functionally innervated (Gray et al., 2013). Interestingly, a subpopulation of bladder interstitial cells that express PDGFRα was also found to express SK3 channels and exhibit currents larger than those of detrusor smooth muscle cells (Lee et al., 2013, 2014). These channels are also active at physiological membrane potentials in interstitial cells, suggesting that SK channels located on these cells may be more responsible for mediating the sensitivity of the bladder to SK channel modulators than those on smooth muscle cells (Lee et al., 2013). Our experimental data support this possibility: for both natural and simulated TCs, apamin increased the gain of the relationship between TC leading slope and afferent activity, such that slower rising TCs generated greater afferent nerve activity (Figs. 4 and 7). Conversely, SK channel activation also reduced the gain of this relationship (Fig. 7). BK channel inhibition affected naturally occurring TCs and their associated afferent nerve activity, but had no effect on afferent activity during simulated TCs (Fig. 7). This suggests that (a) the effects of BK channel inhibition are only attributable to changes in smooth muscle excitability and (b) SK channels regulate a smooth muscle–independent mechanism that limits the maximal amount of sensory output that can be generated during a TC. Inhibition of SK or BK channels did not affect baseline afferent activity (Fig. 5), indicating that this mechanism only modulates the afferent response to TCs. These findings suggest that a subpopulation of interstitial cells are somehow functionally linked to afferent nerves that specifically transmit sensory information during TCs. Although no direct connection between interstitial cells and sensory nerves has been demonstrated, PDGFRα-positive interstitial cells are associated with nerve varicosities in detrusor muscle (Koh et al., 2012). Thus, it is possible that PDGFRα-positive interstitial cells or other populations of interstitial cells mediate communication between TCs and afferent nerves to regulate TC-induced sensory outflow from the bladder.

Summary

TCs generate bursts of afferent nerve activity that greatly outweigh the impact of baseline changes in pressure and cause the majority of sensory nerve outflow from the UB. The impact of TCs on afferent nerve activity increases with filling pressure, suggesting that TCs have an important role in the communication of bladder fullness to the CNS. The TC rate of rise determines the ensuing afferent nerve activity, saturating at ∼3 mmHg/s and 600–800 Hz. Simulation of TCs in the absence of smooth muscle contractility by gentle compression of the bladder wall yielded similar bursts of afferent nerve activity. SK channel inhibition, in the presence and absence of smooth muscle contractile function, increased the amplitude of afferent nerve activity elicited by both naturally occurring and simulated TCs. This suggests the operation of a smooth muscle–independent mechanism,

regulated in part by SK channels, that modulates sensory outflow from the UB. Our results support this idea, implicating SK channels in regulating the excitability of pressure-transducing cells within the bladder wall and thereby impacting the transduction of TCs to afferent nerve activity.

Collectively, our findings show that TCs are responsible for a predominant share of bladder afferent sensory output, especially relative to steady increases in intravesical pressure (Fig. 1 B). This study is also the first to show that afferent nerve activity is strongly coupled to rapid TCs of detrusor smooth muscle. As shown in Fig. 8, the rate of rise of these TCs may be indicative of the length-tension relationship of the bladder smooth muscle and thus serves to transmit information regarding optimal wall tension for maximal and efficient voiding contractions. Importantly, any alteration in bladder wall structure or distensibility (e.g., hypertrophy, fibrosis, and collagen deposition) would affect this length-tension relationship and ultimately alter sensory nerve outflow from the UB. Although the exact nature of the bladder pressure transducer remains elusive, we propose that non–smooth muscle cells within the bladder wall (e.g., PDGFRα-positive interstitial cells) regulate the maximal afferent nerve activity generated in response to a TC and further suggest that the excitability of these cells is regulated by SK channels and not BK channels. This mechanism serves to mitigate the transduction of aberrant sensory information regarding bladder fullness to the CNS by controlling the extent to which changes in detrusor excitability can augment peak afferent nerve activity. More importantly, our findings are the first to uncover the key relationship between the rate of rise of transient detrusor smooth muscle contractions and afferent signaling. Our results strongly support the concept that TCs represent a novel target for therapeutic intervention in UB dysfunction. Decreasing the ability of TCs to trigger afferent nerve bursts, decreasing the frequency of TCs, or decreasing the TC rate of rise by limiting detrusor smooth muscle excitability could be effective means for treating some forms of UB dysfunction.

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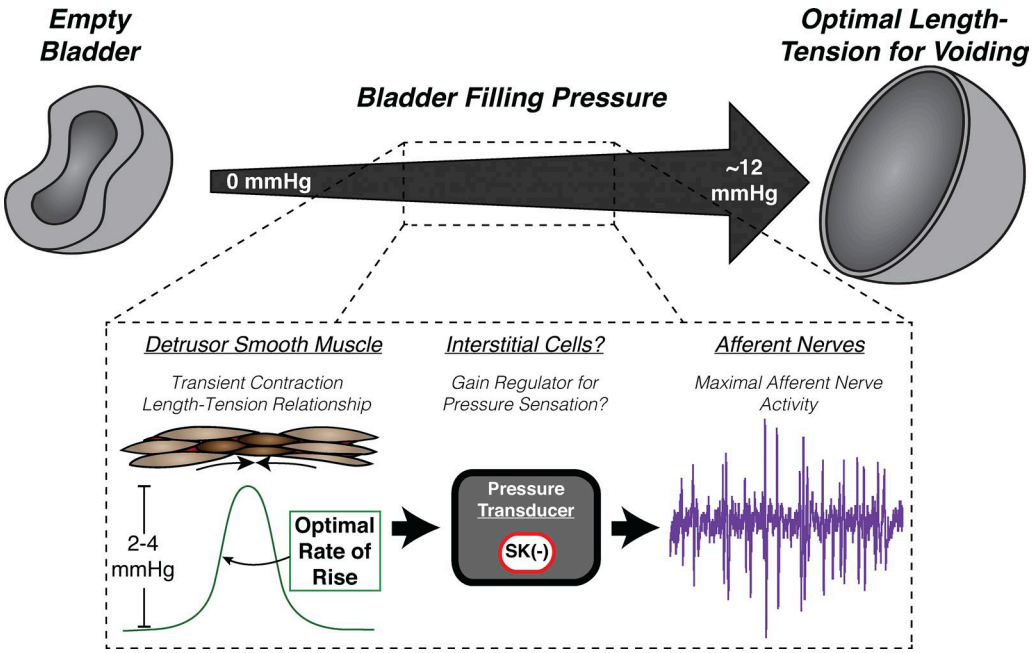

Figure 8. Proposed model of TC-evoked afferent bursts. As the bladder fills, rapid TCs occur. The rate of rise of these TCs is a function of the length-tension relationship of detrusor smooth muscle. TCs stimulate bursts of afferent nerve activity that increase with the rate of rise of the TCs and saturate at ∼3 mmHg/s. The peak afferent activity and maximal TC rate of rise both occur when intravesical pressure is near threshold (∼12 mmHg), which may be indicative of the optimal length-tension relationship for voiding contractions. For simulated and naturally occurring TCs, the SK blocker apamin increased the gain of the relationship between TC leading slope and afferent activity (Fig. 7 D). The molecular identity of the bladder pressure transducer is unknown.

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