Regulation of urinary bladder smooth muscle contractions by ryanodine receptors and BK and SK channels

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The urinary bladder has two important functions: the storage and micturition of urine. These tasks involve a complex integration of signals from sensory systems located in the bladder wall, as well as autonomic reflexes, voluntary regulation of efferent pathways to bladder, and changes in the contractile state of the smooth muscle that makes up the wall of the urinary bladder (for review, see Refs. 2, 5). To understand urinary bladder smooth muscle (UBSM) function, we sought to elucidate fundamental mechanisms underlying excitation-contraction (E-C) coupling in this tissue. UBSM exhibits spontaneous action potentials (2, 9). This activity appears to be related to the phasic nature of spontaneous contractions in this tissue. Phasic contractions of UBSM depend on Ca$^{2+}$ entry through dihydropyridine-sensitive, voltage-dependent Ca$^{2+}$ channels (VDCC) (2). Ca$^{2+}$ entry through VDCC is elevated by the depolarization that occurs during each action potential. Indeed, the upstroke of the action potential is caused by Ca$^{2+}$ entry through VDCC (9). These action potentials are ~20 ms in duration and occur in bursts (9). Two types of Ca$^{2+}$-activated K$^{+}$ (K$_{Ca}$) channels are involved in the restoration of resting membrane potential after a spike. The repolarization of the action potential depends in part on activation of a BK channel, acting as a negative feedback regulator of contraction frequency and duration and thereby determines the onset and length of the interval between contractions.

Despite the importance of spontaneous phasic contractions to urinary bladder function, little is known about the interactions among major components of E-C coupling in this tissue. UBSM action potentials and phasic contractions clearly depend on Ca$^{2+}$ entry through VDCC. The contribution of CICR through RyRs to the phasic contractions, however, is not known, although single cell work suggests a significant contribution of this mechanism (see Ref. 8). In tonic arterial smooth muscle, Ca$^{2+}$ release through RyRs regulates BK channels, acting as a negative feedback regulator of the release of Ca$^{2+}$ from the sarcoplasmic reticulum (SR) that is induced by Ca$^{2+}$ entry [Ca$^{2+}$-induced Ca$^{2+}$ release (CICR)]. The duration of phasic contractions should depend on the duration of the bursts of action potentials. The frequency of phasic contractions should reflect mechanisms that temporarily cause action potentials to cease. Because the action potentials depend on Ca$^{2+}$ entry, accumulated inactivation of VDCC or deactivation of VDCC by membrane hyperpolarization would lead to a cessation of action potentials and thereby determine the onset and length of the interval between contractions.

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element to decrease Ca$^{2+}$ entry (14, 18). The roles of SK and BK channels in the regulation of phasic contractions are unresolved.

The goal of the present study was to determine the roles of RyRs, BK channels, and SK channels in the regulation of phasic contractions of UBSM. Our results indicate that RyRs in UBSM, unlike cardiac muscle, do not elevate contractility through Ca$^{2+}$ release. In fact, RyRs appear to reduce overall force production by decreasing the frequency of phasic contractions. BK and SK channels regulate the frequency, amplitude, and duration of the phasic contractions. Our results suggest important indirect interactions among RyRs and BK and SK channels to regulate UBSM phasic contractions.

**METHODS**

**General.** All procedures were reviewed and approved by the Office of Animal Care Management at the University of Vermont. Guinea pigs (250–350 g) were euthanized by halothane overdose followed by exsanguination. The urinary bladder was removed and placed in ice-cold physiological saline solution (PSS; see **Solutions** for composition). After this rinse in PSS, the bladder was pinned to the bottom of a Sylgard-coated Petri dish containing nominally Ca$^{2+}$-free dissection solution (see below). After the surrounding adipose and connective tissues were removed, the bladder was cut open with a longitudinal incision beginning from the urethral orifice. The mucosal surface of the bladder was washed repeatedly with dissection solution until remaining traces of urine were removed. The bladder was pinned serosal side up for dissection. Individual bundles of detrusor muscle (100- to 300-μm wide) were cut free from the bladder wall and transferred to a small Petri dish containing dissection solution. Miniature aluminum clips were placed at each end of the muscle strip to allow mounting of the strip in a tissue bath. Individual strips were placed in water-jacketed tissue baths (2 ml volume) maintained at 37°C. One end of the strip was attached to a stationary metal hook while the other end was connected to a force-displacement transducer (model BG-10G; Kulite Semiconductor Products) for measuring isometric contractility. Urinary bladder strips were equilibrated at connected to a stationary metal hook while the other end was muscle strip to allow mounting of the strip in a tissue bath. Miniature aluminum clips were placed at each end of the strip. To elicit neurally mediated contractions, strips were subjected to electrical field stimulation to trigger nerve activity and elicit contractile responses. To verify that this cocktail was effective at inhibiting neurotransmitter receptors in the bladder wall (atropine, muscarinic antagonist; phentolamine, α-adrenergic antagonist; propranolol, β-adrenergic antagonist; suramin or α,β-methylene ATP, purinergic antagonist; tetrodotoxin, neuronal Na$^+$ channel blocker). Spontaneous contractions were observed within 20–30 min of equilibration and were elevated. SP82

**Field stimulation.** To rule out the possibility that responses examined in this study were due to changes in the activity of nerves contained in the bladder strips, all experiments were conducted in the presence of a cocktail containing tetrodotoxin, as well as receptor antagonists for neurotransmitters in the bladder (Ref. 6; see **Solutions** for composition). To confirm that this cocktail was effective at inhibiting neurally mediated responses, some strips were subjected to electric field stimulation to trigger nerve activity and elicit UBSM contraction in the presence and absence of the cocktail. For these experiments, UBSM strips were mounted in a modified organ chamber equipped with silver stimulating electrodes. To elicit neurally mediated contractions, strips were stimulated for 2 s, using a 0.3-ms pulse of 20–40 V at 20 Hz (model S-88, Grass Instruments). Responses from three successive stimulations were averaged for each strip, before and after exposure to the cocktail.

**Solutions.** PSS was made daily and contained (in mM) 119 NaCl, 4.7 KCl, 24 NaHCO$_3$, 1.2 KH$_2$PO$_4$, 2.5 CaCl$_2$, 1.2 MgSO$_4$, 0.023 ethylenediaminetetraacetic acid, and 11 glucose and was aerated with 95% O$_2$-5% CO$_2$ to obtain pH 7.4. Dissection solution contained (in mM) 80 monosodium glutamate, 55 NaCl, 6 KCl, 10 glucose, 10 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid, 2 MgCl$_2$, pH adjusted to 7.3 with NaOH. Iberiotoxin and apamin (both from Sigma) were prepared as stock solutions in distilled water. Ryanodine (L. C. Laboratories) was prepared in dimethyl sulfoxide. The cocktail containing neurotransmitter antagonists was prepared directly in PSS and contained (in μM) 1 atropine, 1 phentolamine, 1 propranolol, 1 tetrodotoxin, and either 10 suramin or 1 α,β-methylene ATP (all from Sigma). Nisoldipine was prepared in ethanol.

**Calculations and statistics.** Summary data are presented as means ± SE from n separate preparations. Force integral is calculated by integrating the area under the force-time curve for a period of 5 min. Contractile frequencies, amplitudes, and force integrals are expressed relative to control (absence of pharmacological intervention) values. In all protocols, test agents were applied for 15 min, with the last 5 min taken as the analysis period. Control data were obtained in the 5 min preceding the first test compound. Differences were assessed by t-test or one-way analysis of variance, where appropriate, and the null hypothesis was rejected for all P < 0.05. The Student-Newman-Keuls method was used for all pairwise multiple comparisons.

**RESULTS**

**Myogenic nature of spontaneous contractions in UBSM.** Smooth muscle from the urinary bladder is known to exhibit spontaneous electrical excitability in the form of action potentials (2, 9), resulting in rhythmic contractile activity. We sought to investigate the role of ryanodine-sensitive Ca$^{2+}$ release and BK and SK channels in modulating spontaneous contractions of guinea pig UBSM. Baseline contractile variables for all muscle strips used in this study are summarized in Table 1. To verify that spontaneous contractility in this preparation is not caused by neurotransmitters released from autonomic nerves contained in the bladder strips, experiments were performed in the presence of a cocktail containing blockers for known transmitter receptors in the bladder wall (atropine, muscarinic antagonist; phentolamine, α-adrenergic antagonist; propranolol, β-adrenergic antagonist; suramin or α,β-methylene ATP, purinergic antagonist; tetrodotoxin, neuronal Na$^+$ channel blocker). Spontaneous contrac-

| Table 1. Baseline contractile variables for UBSM strips in the presence of drug cocktail |
|----------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Resting Load, mN | Frequency, Contractions/min | Amplitude, mN | Duration, s | Integrated Force, mN/s |
| 1.15 ± 0.04 | 3.1 ± 0.4 | 0.68 ± 0.09 | 8.4 ± 0.3 | 27.0 ± 3.9 |

Values are means ± SE. All measurements were taken during a 5-min period before administration of pharmacological agents (n = 37 separate strips used in the present study). Integrated force is the cumulative area under the force-time curve for the entire analysis period. UBSM, urinary bladder smooth muscle.
2.1 to 1.6

6

6

activity. Contraction frequency decreased (from 4.8 contractions (Fig. 1 cocktail, suggesting a myogenic basis for these contractions of UBSM persisted in the presence of a cocktail containing (in μM) 1 atropine, 1 phenolamine, 1 propranolol, 1 tetrodotoxin, and 10 suramin. The insets show selected regions of the record on an expanded time scale (same in both insets) for clarity. The vertical scale in the insets is the same as the main trace. B: nerve-evoked contractions are abolished by the cocktail. Each trace shows a contractile response induced by field stimulation (30-V pulses of 0.3-ms duration at 20 Hz for 2 s) in a single UBSM strip before application of the cocktail (Control), after 10-min incubation in the cocktail (Cocktail), and after 20 min of washout (Wash). Stimulation occurred at the time indicated by the vertical arrow. The original records shown are characteristic of 5 strips. C: representative trace showing that spontaneous contractions (in the presence of cocktail) are completely abolished after inhibition of VDCC with nisoldipine. D-F: summary data showing the effects of the cocktail on frequency, amplitude, and force integral in UBSM strips. *P < 0.05 vs. control.

To ensure that neuronally mediated contractions could be blocked by the cocktail, nerve responses were elicited in one group of strips using electric field stimulation (0.3-ms pulses of 20–40 V at 20 Hz for 2 s; Fig. 1B). Before application of the cocktail, the average amplitude of the field stimulation-evoked contractions was 3.14 ± 0.96 mN with a duration of 4.8 ± 0.9 s (Fig. 1B “Control”, n = 5). After a 10-min incubation in the cocktail, the field stimulation-evoked contractions were completely abolished (Fig. 1B “Cocktail”). Furthermore, the cocktail-induced inhibition of these contractions was reversible, as contractions could be evoked by field stimulation after ~20 min of washout with PSS (Fig. 1B “Wash”). After washout, field stimulation-evoked contraction amplitude was 1.43 ± 0.71 mN (~46% of control), and the duration was 2.7 ± 0.4 s (~56% of control; n = 3). Thus the cocktail was effective at inhibiting nerve-mediated responses in UBSM, and all experiments performed in the remainder of this study were conducted in the presence of the drug cocktail to minimize the contribution of neurogenic responses after pharmacological interventions.

Phasic contractions require Ca^{2+} entry through VDCC. To examine the role of Ca^{2+} influx through VDCC in mediating UBSM contractions, the dihydropyridine inhibitor of VDCC, nisoldipine (0.1–1.0 μM), was administered to spontaneously contracting strips. Nisoldipine inhibited contractile activity (Fig. 1C; n = 5). In five strips studied, the average time for complete inhibition of contractions by nisoldipine was 2.2 ± 0.5 min. This finding indicates that Ca^{2+} influx through VDCC is essential for spontaneous contractile events.

RyRs modulate the frequency of phasic contractions. Ca^{2+} release from RyRs on the SR is thought to be involved in E-C coupling in UBSM (7, 8, 11, 12). We hypothesized that Ca^{2+} release from RyRs (CICR) contributes a portion of the Ca^{2+} stimulus required for cell contraction, resulting in an increase in amplitude of UBSM contractions. To examine the functional role for SR Ca^{2+} release via RyRs, we treated contracting UBSM strips with ryanodine (10 μM) to inhibit RyRs (Fig. 2). This concentration of ryanodine was confirmed to inhibit RyR-mediated Ca^{2+} release (Ca^{2+} sparks) and their associated spontaneous transient outward currents in guinea pig UBSM cells (10; see also Refs. 8, 13, 18, 20). The effects of ryanodine were assessed 10–15 min after application to ensure a steady-state effect. Application of ryanodine (10 μM) resulted in an
increase in contraction frequency from $2.9 \pm 0.4$ to $4.8 \pm 1.2$ contractions/min (Fig. 2B), but no significant change in contraction amplitude ($0.97 \pm 0.13$ vs. $1.07 \pm 0.25$ mN; Fig. 2C). The contraction duration was also unaffected by ryanodine ($8.9 \pm 0.4$ vs. $9.8 \pm 0.5$ s; $P = 0.16$). Therefore, contrary to the expectation that ryanodine would reduce force by 70% (see Ref. 12), ryanodine actually increased integrated force (from $37.3 \pm 4.0$ to $65.6 \pm 13.4$ mN/s; Fig. 2D), reflecting the increase in contraction frequency. These results suggest that RyR-mediated Ca$^{2+}$ release acts to decrease, and not increase, overall contractility. Possible mechanisms by which RyR-mediated Ca$^{2+}$ release could decrease contractility are through activation of BK and SK channels.

Inhibition of BK or SK channels increases the amplitude and duration, but decreases the frequency, of UBSM contractions. The role of BK channels in UBSM contractility was tested by applying the potent and selective BK channel blocker iberiotoxin (100 nM, ~100 times inhibition constant, see Refs. 6a and 19). Blocking BK channels dramatically increased contraction amplitude (from $0.32 \pm 0.06$ to $1.27 \pm 0.45$ mN; Fig. 3C), duration (from $6.2 \pm 0.8$ to $16.1 \pm 5.6$ s; Fig. 3D), and integrated force (from $12.6 \pm 4.2$ to $57.3 \pm 22.7$ mN/s). The amplitude of the phasic contractions in the presence of iberiotoxin was comparable to the force produced in response to 60 mM extracellular K$^+$ concentration ([K$^+$], $0.80 \pm 0.13$ mN, $n = 7$). Iberiotoxin also decreased contraction frequency by 53% from $4.4 \pm 1.3$ to $2.0 \pm 0.5$ contractions/min (Fig. 3B). The reduction of contraction frequency to iberiotoxin appeared to require functional RyRs, because iberiotoxin (100 nM) did not reduce contraction frequency in the presence of 10 $\mu$M ryanodine ($3.7 \pm 0.4$ in ryanodine vs. $5.3 \pm 1.5$ contractions/min in ryanodine and iberiotoxin; $n = 6$). These observations indicate that BK channels play a profound role in the regulation of UBSM contractility.
In the presence of iberiotoxin, ryanodine did not change contraction amplitude (Fig. 3C) or duration (Fig. 3D). However, ryanodine did increase contraction frequency, as it did in the absence of iberiotoxin (66 and 99% increase in frequency in the absence and presence of iberiotoxin, respectively; Fig. 3B, see also Fig. 2B). These results suggest that BK channels are not required for frequency modulation of UBSM contractility by RyRs. Furthermore, these results do not support a direct role for RyR-mediated \( \text{Ca}^{2+} \) release causing contraction, because ryanodine did not reduce the amplitude of contraction, even after enhancing \( \text{Ca}^{2+} \) entry through iberiotoxin-induced increases in action potential duration and frequency (9) and contractility (Fig. 3).

The role of SK channels was examined by testing the effects of apamin (100 nM; Fig. 4), a potent and selective blocker of SK2 and SK3 channels (16). Blocking SK channels reduced contraction frequency (from 2.3 ± 0.3 to 1.6 ± 0.4 contractions/min; Fig. 4B) while increasing the contraction amplitude (from 0.70 ± 0.20 to 1.20 ± 0.25 mN; Fig. 4C) and integrated force (from 22.1 ± 4.2 to 28.8 ± 4.6 mN/s; \( P < 0.05 \)). Contraction duration also increased significantly after inhibition of SK channels (from 9.1 ± 0.6 to 12.8 ± 1.5 s; Fig. 4D). This observation is consistent with the idea that enhanced SK channel activity during the afterhyperpolarization acts to decrease excitability and, thereby, contraction. Preventing the afterhyperpolarization by blocking SK channels should lead to an increase in action potential frequency, enhanced \( \text{Ca}^{2+} \) entry through VDCC, and a subsequent increase in contraction amplitude and duration.

Blocking SK channels unmask a role for RyRs in modulating contraction duration. Ryanodine (10 µM) by itself did not cause a steady-state effect on contraction amplitude or duration (Fig. 2). However, in the presence of apamin, ryanodine increased contraction duration (from 12.8 ± 1.5 to 21.3 ± 5.9 s; Fig. 4D) and the apamin-induced increase in contraction amplitude persisted (Fig. 4C). The net effect of ryanodine, in the presence of apamin, led to a 3.2-fold increase in integrated force (from 28.8 ± 4.6 to 92.9 ± 27.1 mN/s), compared with 1.8-fold increase in the absence of apamin (see Fig. 2).

**SK channel modulation of contraction amplitude and duration is enhanced by RyR inhibition.** Apamin alone increased contraction amplitude, duration, and integrated force (Fig. 4). Because blocking SK channels unmasked a role for RyRs in regulating UBSM contraction duration (above), the effect of blocking SK channels in the presence of ryanodine was examined (Fig. 5). The presence of ryanodine did not alter the basic responses to apamin: an increase in amplitude, duration, and integrated force. However, the increases in both contraction amplitude and duration to apamin were significantly greater in the presence of ryanodine than in the absence (compare Fig. 4, C and D, to Fig. 5, C and D). Apamin alone caused a 1.8 ± 0.2-fold increase in contraction amplitude, whereas in the presence of ryanodine (which did not affect amplitude), apamin increased amplitude by 2.7 ± 0.2-fold (\( P = 0.02 \)). The apamin-induced change in contraction duration increased from 1.4 ± 0.1-fold over control to 4.3 ± 2.1-fold over control in the absence and presence of ryanodine, respectively (\( P = 0.01 \)). Furthermore, the increase in integrated force elicited by apamin in the presence of RyR inhibition was substantially greater than that caused by apamin alone (4.0 ± 1.1- vs. 1.5 ± 0.2-fold, \( P = 0.01 \)). The striking augmentation of apamin-induced changes in UBSM contractility by ryanodine can be seen in Fig. 5E. This figure illustrates original recordings of contractions from two separate UBSM strips (strips A and B). A single contraction (representing the average) from each strip is shown under control conditions (No Drug) to show that basal contractility was similar between the two strips. Finally, a single contraction from each strip is shown after apamin treatment, either in the presence or absence of ryanodine. Note that ryanodine pretreatment substantially augmented the increase in contraction amplitude and duration induced by apamin. This find-
ing suggests that Ca\textsuperscript{2+} release through RyRs acts to dampen the effects of SK channel inhibition.

**DISCUSSION**

In this study, we explored the roles of VDCC, RyRs, and BK and SK channels in the regulation of phasic contractions of UBSM. Phasic contractions of UBSM depend on Ca\textsuperscript{2+} entry through dihydropyridine-sensitive VDCC (Fig. 1; see Ref. 2). Ca\textsuperscript{2+} entry is activated during action potentials, with the upstroke of the action potential also depending on Ca\textsuperscript{2+} currents through VDCC (2, 9). Because action potentials are brief (20 ms) with respect to phasic contractions (3–10 s), a phasic contraction presumably reflects a burst of action potentials. Our results indicate that RyRs, BK channels, and SK channels all play important roles, as negative feedback elements, to regulate urinary bladder contractility (Fig. 6).

**Inhibition of RyRs does not decrease the amplitude of the phasic contractions: no evidence for “classical” CICR.** In cardiac muscle, RyRs are activated by Ca\textsuperscript{2+} entry during the action potential to cause massive Ca\textsuperscript{2+} release (CICR) that contributes the majority of the Ca\textsuperscript{2+} (90%) during the Ca\textsuperscript{2+} transient (4). Evidence for a similar mechanism in UBSM has come from experiments on voltage-clamped isolated smooth muscle cells from urinary bladder. The incremental rise in intracellular Ca\textsuperscript{2+} concentration ([Ca\textsuperscript{2+}]\textsubscript{i}) during depolarization of UBSM is greater than would be expected through VDCC, which occurs during a burst of action potentials, determines contractile strength. Spontaneous contractility is regulated by both BK and SK channels, which limit Ca\textsuperscript{2+} entry as a result of action potential repolarization and afterhyperpolarization, respectively. BK and SK channel activity could be increased by local Ca\textsuperscript{2+} entry through VDCC or by depolarization (BK channels). Ca\textsuperscript{2+} release from RyRs does not directly regulate contraction amplitude, but rather is coupled to some other Ca\textsuperscript{2+}-dependent process to regulate contraction frequency in a negative-feedback manner. Potential targets for RyR-mediated Ca\textsuperscript{2+} release include BK and SK channels, as well as VDCC (Ca\textsuperscript{2+}-dependent inactivation). RyR-mediated Ca\textsuperscript{2+} release may be stimulated by Ca\textsuperscript{2+} entry through VDCC (CICR), but Ca\textsuperscript{2+} released in this manner does not contribute to spontaneous contractility.

**Fig. 5.** Apamin-induced increases in contraction amplitude and duration are augmented by ryanodine pretreatment. A: original record showing the effect of blocking SK channels with apamin in the presence of ryanodine. B: apamin decreased contraction frequency to 40% of control in the presence of RyR inhibition. C: ryanodine did not significantly alter contraction amplitude, but inhibition of SK channels increased UBSM contraction amplitude by ~2.5-fold. D: inhibiting RyRs did not change contraction duration, whereas apamin increased contraction duration ~4-fold over control. E: representative individual contractions from 2 separate UBSM strips showing contractions under resting conditions (No Drug) and after inhibition of SK channels with apamin (100 nM) in the presence (shaded trace, strip A) or absence (black trace, strip B) of ryanodine (10 μM). Note that the response to apamin is greatly augmented after ryanodine pretreatment. The contractions from strip A were taken from the recording shown in A, above ●. *P < 0.05 vs. control. †P < 0.05 vs. ryanodine.

**Fig. 6.** Proposed interactions among RyRs, BK channels, SK channels, and VDCC in regulating UBSM contractility. Ca\textsuperscript{2+} entry through VDCC, which occurs during a burst of action potentials, determines contractile strength. Spontaneous contractility is regulated by both BK and SK channels, which limit Ca\textsuperscript{2+} entry as a result of action potential repolarization and afterhyperpolarization, respectively. BK and SK channel activity could be increased by local Ca\textsuperscript{2+} entry through VDCC or by depolarization (BK channels). Ca\textsuperscript{2+} release from RyRs does not directly regulate contraction amplitude, but rather is coupled to some other Ca\textsuperscript{2+}-dependent process to regulate contraction frequency in a negative-feedback manner. Potential targets for RyR-mediated Ca\textsuperscript{2+} release include BK and SK channels, as well as VDCC (Ca\textsuperscript{2+}-dependent inactivation). RyR-mediated Ca\textsuperscript{2+} release may be stimulated by Ca\textsuperscript{2+} entry through VDCC (CICR), but Ca\textsuperscript{2+} released in this manner does not contribute to spontaneous contractility.
if VDCC were the only source of Ca$^{2+}$ (12). Thus it has been suggested that the Ca$^{2+}$ influx through VDCC is amplified by Ca$^{2+}$ released from RyRs in the SR via CICR (8, 12). Indeed, voltage-activated Ca$^{2+}$ currents evoked Ca$^{2+}$ transients that were reduced by ~70% by ryanodine (8), suggesting that substantial Ca$^{2+}$ for the transient was derived from RyR-mediated Ca$^{2+}$ release. However, there are notable differences between CICR in cardiac myocytes and in UBSM. CICR in cardiac myocytes is very rapid (<5 ms; see Ref. 4) because of the close apposition of VDCC and RyRs. In sharp contrast, CICR in isolated urinary bladder myocytes develops over hundreds of milliseconds, arguing against a close proximity of VDCC and RyRs in this preparation. On the basis of these measurements, “cardiac muscle-like” CICR in urinary bladder is arguably too slow to be activated by normally occurring action potentials, which have a duration of 20 ms. Ca$^{2+}$ entry through VDCC during the action potential of UBSM could activate RyRs through an elevation of cytoplasmic Ca$^{2+}$ and SR Ca$^{2+}$ load.

In cardiac and skeletal muscle, the major role of RyRs is to deliver Ca$^{2+}$ for contraction. In UBSM, our results indicate that the major role of RyRs is to decrease contraction. RyR-mediated Ca$^{2+}$ release could decrease excitability through activation of K$^+$ channels or inhibition of inward currents such as the VDCC. Ca$^{2+}$-mediated inactivation of VDCC would not only decrease excitability, but would also directly decrease Ca$^{2+}$ entry and, thus, contractility. Our results suggest that the direct contribution of RyR-mediated Ca$^{2+}$ release to average cytosolic Ca$^{2+}$ concentration via “cardiac-like CICR” is small compared with its ability to decrease excitability (Fig. 6). Our approach precludes a direct estimate of the RyR-mediated Ca$^{2+}$ release. However, our results suggest that RyR-mediated Ca$^{2+}$ release in UBSM is under dynamic control from a number of negative feedback loops (Fig. 6). An approach to assessing the amount of RyR-mediated Ca$^{2+}$ release would be to measure [Ca$^{2+}$]$_i$ in voltage-clamped myocytes subjected to simulated action potentials. The effect of this [Ca$^{2+}$]$_i$ on VDCC inactivation would also have to be evaluated.

**Role of RyRs in the regulation of phasic contractions.**

RyR-mediated Ca$^{2+}$ release acts to limit UBSM contraction frequency. Thus blocking RyRs increases contraction frequency (Fig. 2). An increase in contraction frequency to blocking RyRs could reflect an elevation of excitability, resulting from either less hyperpolarization or less inactivation of VDCC during the quiescent phase between action potential bursts. Obvious targets of RyR-mediated Ca$^{2+}$ release that would decrease excitability and, thereby, decrease contraction frequency are SK and BK channels. In the presence of the BK channel blocker iberiotoxin, ryanodine still increased contraction frequency (Fig. 3). In the presence of the SK channel blocker apamin, contraction frequency decreased (Fig. 4) and remained decreased after ryanodine (Fig. 4). These results point to a role of SK channels in ryanodine-induced increase in contraction frequency.

The negative feedback regulation of contractility by RyRs is readily apparent in the presence of apamin (Figs. 4 and 5). Ryanodine-induced elevation of contraction duration in the presence of apamin is consistent with the idea that RyRs play a major role as a negative feedback element to limit contractility (Fig. 6). It is conceivable that in the absence of apamin, RyR-mediated Ca$^{2+}$ release regulates UBSM contractility through activation of BK channels. This possibility is difficult to test, because the combined treatment of apamin and iberiotoxin led to large chaotic contractions, precluding any interpretation of the effect of ryanodine in the presence of iberiotoxin and apamin.

The interplay of RyRs with BK channels is more difficult to interpret at the level of contractions. Iberiotoxin-induced contractions were comparable in amplitude to those observed in high potassium. Therefore, it is unlikely that the addition of ryanodine or apamin could increase contraction amplitude in the presence of iberiotoxin. Iberiotoxin increased amplitudes and durations in the presence of ryanodine, suggesting that BK channels can be activated in the absence of RyR-mediated Ca$^{2+}$ release. During the action potential, the membrane depolarization and subsequent elevation of [Ca$^{2+}$]$_i$ are apparently sufficient to cause a significant activation of BK channels. The present study does not directly address the role of local RyR-mediated Ca$^{2+}$ release (Ca$^{2+}$ sparks) in the activation of BK channels, which does occur in this preparation (10). This element may also be involved in the negative feedback regulation of excitability by RyRs (Fig. 6).

**BK channels play a profound role in the regulation of UBSM contractility.**

Blocking BK channels caused a pronounced increase in the amplitude and duration of the phasic contractions. This response was somewhat offset by a decrease in contraction frequency (Fig. 3). However, the net effect of blocking BK channels was a substantial increase in average force. The increase in contraction amplitude and duration to iberiotoxin was not affected by ryanodine, suggesting that BK channels contribute to regulation of contractility in the absence of SR Ca$^{2+}$ release through RyRs. Furthermore, the increase in contraction amplitude and duration to iberiotoxin was not affected by ryanodine, suggesting that BK channels contribute to regulation of contractility in the absence of SR Ca$^{2+}$ release through RyRs. Furthermore, the increases in contraction amplitude and duration to iberiotoxin was not affected by ryanodine, suggesting that BK channels contribute to regulation of contractility in the absence of SR Ca$^{2+}$ release through RyRs. Furthermore, the increases in contraction amplitude and duration to iberiotoxin was not affected by ryanodine, suggesting that BK channels contribute to regulation of contractility in the absence of SR Ca$^{2+}$ release through RyRs. Furthermore, the increases in contraction amplitude and duration to iberiotoxin was not affected by ryanodine, suggesting that BK channels contribute to regulation of contractility in the absence of SR Ca$^{2+}$ release through RyRs.

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Ca²⁺ itself and, depending on voltage, may require >10 μM Ca²⁺ for maximal activity.

It is possible that the decrease in contraction frequency to iberiotoxin reflects a Ca²⁺-dependent process, because [Ca²⁺], is presumably greatly elevated during the phasic contractions in the presence of iberiotoxin. Increased [Ca²⁺], during a phasic contraction would lead to greater SR Ca²⁺ load and presumably more Ca²⁺ release. The effects of ryanodine on the iberiotoxin-induced changes in contraction amplitude, duration, and frequency were examined. Ryanodine did not prevent the iberiotoxin-induced increase in amplitude or duration, but it did prevent the decrease in contraction frequency. This result suggests that RyR-mediated Ca²⁺ release can slow the contraction frequency, consistent with the observation that blocking RyRs can increase contraction frequency.

SK channels regulate the amplitude and frequency of UBSM contractions. SK channels represent another important target for [Ca²⁺], and they may contribute to regulation of UBSM excitability. These channels have been best characterized in hippocampal neurons (16, 17, 24) where they underlie the slow afterhyperpolarization (21). Apamin-sensitive SK channels have not been extensively characterized in smooth muscle (15, 22), although there is considerable functional evidence suggesting their importance in mediating the afterhyperpolarization in UBSM (2, 6). We examined the role of apamin-sensitive SK channels in regulating UBSM contractility. Our results suggest that SK channels contribute to regulating UBSM contractility, because apamin increased contraction amplitude and force production but decreased contraction frequency. Furthermore, in the presence of ryanodine, the contractile response to apamin was substantially augmented (Fig. 5). One possible interpretation is that SK channels directly sense local Ca²⁺ influx through VDCC, as has been suggested for these channels in hippocampal neurons (17). Ca²⁺ influx through VDCC could increase in the presence of ryanodine, owing to the relief of Ca²⁺-dependent inactivation of VDCC (1). The increased Ca²⁺ influx would lead to greater activation of SK channels. This augmented contribution of SK channels to regulating contractility is unmasked when SK channels are inhibited in the presence of ryanodine.

In conclusion, the present study provides the first characterization of the effects of inhibitors of RyRs (ryanodine), BK channels (iberiotoxin), and SK channels (apamin) on phasic UBSM contractions. BK channel inhibition causes such profound increases in contraction amplitude and duration that neither elevation of SK channel activity nor RyRs could compensate. Inhibition of RyRs results in modulation of contraction frequency. However, combined SK and RyR inhibition leads to an increase in both contraction amplitude and duration. The results indicate that RyRs, BK channels, and SK channels function as negative-feedback elements through differential modulation of contractile amplitude, duration, and frequency (Fig. 6).

Perspectives

Several issues of key importance stem from this work. The spontaneous nature of phasic contractions in guinea pig UBSM is a characteristic that may have substantial use in understanding human bladder function. Specifically, these phasic contractions in guinea pig detrusor resemble the spontaneous contractility that is seen in human detrusor (see Ref. 3). Furthermore, a hallmark of the unstable bladder, both human and animal models, is hyperexcitability of the smooth muscle (3). Thus understanding how electrical events are coordinated and regulated to cause contraction in guinea pig bladder should provide insights into dysregulation of E-C coupling in human bladder disease. By elucidating key ion channels that regulate UBSM contractility, such as SK channels, BK channels, VDCC, and RyRs, it is possible that new targets will be identified for the development of pharmacological agents aimed at treating bladder dysfunction. Furthermore, the nature of the spontaneous rhythmicity characteristic of the guinea pig urinary bladder has been elusive. Our results suggest that guinea pig urinary bladder smooth muscle is capable of generating phasic contractions, independent of nerve input. One possible explanation is that stretch-sensitive cation channels are responsible for generating an excitatory pacemaker potential that depolarizes the myocytes to the point where a VDCC-mediated action potential fires (23). A burst of action potentials, then, is associated with the phasic contraction. Consistent with this hypothesis is the observation that blocking VDCC in guinea pig UBSM abolishes the action potential spike (9) and contractions (Fig. 1), but small oscillatory depolarizations remain (9). The ionic basis for these remaining depolarizations, as well as regulation by RyRs, remains to be elucidated.

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