The reverse mode of the Na\(^+\)/Ca\(^{2+}\) exchanger provides a source of Ca\(^{2+}\) for store refilling following agonist-induced Ca\(^{2+}\) mobilization


Asthma Research Group, Firestone Institute for Respiratory Health, St. Joseph’s Healthcare, and Department of Medicine, McMaster University, Hamilton, Ontario, Canada

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Hirota S, Pertens E, Janssen LJ. The reverse mode of the Na\(^+\)/Ca\(^{2+}\) exchanger provides a source of Ca\(^{2+}\) for store refilling following agonist-induced Ca\(^{2+}\) mobilization. Am J Physiol Lung Cell Mol Physiol 292: L438–L447, 2007. First published October 13; doi:10.1152/ajplung.00222.2006.—Agonist-induced contraction of airway smooth muscle (ASM) can be triggered by an elevation in the intracellular Ca\(^{2+}\) concentration, primarily through the release of Ca\(^{2+}\) from the sarcoplasmic reticulum (SR). The refilling of the SR is integral for subsequent contractions. It has been suggested that Ca\(^{2+}\) entry via store-operated cation (SOC) and receptor-operated cation channels may facilitate refilling of the SR. Indeed, depletion of the SR activates substantial inward SOC currents in ASM that are composed of both Ca\(^{2+}\) and Na\(^+\). Accumulation of Na\(^+\) within the cell may regulate Ca\(^{2+}\) handling in ASM by forcing the Na\(^+\)/Ca\(^{2+}\) exchanger (NCX) into the reverse mode, leading to the influx of Ca\(^{2+}\) from the extracellular domain. Since depletion of the SR activates substantial inward Na\(^+\) current, it is conceivable that the reverse mode of the NCX may contribute to the intracellular Ca\(^{2+}\) pool from which the SR is refilled. Indeed, successive contractions of bovine ASM, evoked by various agonists (ACh, histamine, 5-HT, caffeine) were significantly reduced successive contractions induced by both Ca\(^{2+}\) and Na\(^+\). Oubain, a selective inhibitor of the Na\(^+\)/K\(^+\) pump, had no effect on the reductions observed under normal and zero-Na\(^+\) conditions. KB-R7943, a selective inhibitor of the reverse mode of the NCX, significantly reduced successive contractions induced by both agonists without altering KCl responses. Furthermore, KB-R7943 abolished successive caffeine-induced Ca\(^{2+}\) transients in single ASM cells. Together, these data suggest a role for the reverse mode of the NCX in refilling the SR in ASM following Ca\(^{2+}\) mobilization.

AGONIST-INDUCED CONTRACTION of airway smooth muscle (ASM) can be triggered by an elevation in the intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_{i}\)), primarily through the release of Ca\(^{2+}\) from the sarcoplasmic reticulum (SR). Elevated [Ca\(^{2+}\)]\(_{i}\) is alleviated by pumps including the sarcoplasmic reticulum Ca\(^{2+}\)-ATPase (SERCA) and plasma membrane Ca\(^{2+}\)-ATPase (PMCA), which actively sequester Ca\(^{2+}\) into the SR and/or extrude Ca\(^{2+}\) from the cell, respectively. The resulting Ca\(^{2+}\) deficit following its extrusion must be replaced to adequately refill the SR. It has been suggested that Ca\(^{2+}\) entry via L-type Ca\(^{2+}\) channels, store-operated cation (SOC) channels, and receptor-operated cation (ROC) channels may act to compensate for the loss of Ca\(^{2+}\) resulting from active extrusion (24, 27). Indeed, depletion of the SR activates substantial SOC currents in many smooth muscle types, including ASM (13). In addition to conducting Ca\(^{2+}\), SOC channels, formed by hetero-or homotetrameric arrangements of transient receptor potential (TRP) proteins, conduct a substantial inward Na\(^+\) current (13, 29, 33).

In addition to SERCA and PMCA, many studies have suggested that the Na\(^+\)/Ca\(^{2+}\) exchanger (NCX) is involved in the regulation of [Ca\(^{2+}\)]\(_{i}\), within smooth muscle cells (24, 28, 34). The bidirectional transporter normally moves Ca\(^{2+}\) out of the cell by utilizing the Na\(^+\) concentration gradient across the cell membrane. However, accumulation of Na\(^+\) within the cell can reduce Ca\(^{2+}\) extrusion and even force the NCX into the reverse mode, causing an influx of Ca\(^{2+}\) from the extracellular domain (10, 38) (for review, see Ref. 4). Although the reverse mode of the NCX is well established in cardiac cells, less is known about its role in smooth muscle. In smooth muscle, depletion of the SR activates SOC channels, elevating intracellular Na\(^+\), an event that favors the reverse mode of the NCX (1, 13). Thus it is conceivable that the reverse mode of the NCX may contribute to the intracellular Ca\(^{2+}\) pool from which the SR is refilled following store depletion. Leblanc and Leung (21) described a Na\(^+\)-dependent Ca\(^{2+}\) influx pathway in rabbit portal vein smooth muscle consistent with the reverse mode of the NCX. Arnon et al. (1) reported that Na\(^+\) influx via SOC channels augmented Ca\(^{2+}\) signaling and reduced extrusion of Ca\(^{2+}\) in arterial myocytes. Furthermore, Wu and Fry (38) demonstrated that Na\(^+\)-dependent influx of Ca\(^{2+}\) through the reverse mode of the NCX could contribute to the overall pool of intracellular Ca\(^{2+}\) in guinea pig detrusor smooth muscle. In non-smooth muscle systems, TRP channels and NCX also colocalize and contribute to sustained elevations in [Ca\(^{2+}\)]\(_{i}\), through Na\(^+\)-dependent Ca\(^{2+}\) influx via the reverse mode of the NCX (29, 33).

The aim of our current study was to examine the role of the reverse mode of the NCX in the refilling of the SR in ASM in the context of agonist-induced Ca\(^{2+}\) release in whole tissue and single-cell preparations. Throughout our study we employed a variety of agonists that trigger Ca\(^{2+}\) release by different mechanisms to determine whether the activation of the reverse mode of the NCX was dependent on specific receptor-mediated events or store depletion. Acetylcholine (ACh), histamine, and 5-HT act at different protein-coupled receptors to mobilize Ca\(^{2+}\), whereas caffeine acts directly on the SR. We hypothesized that agonist-induced mobilization of Ca\(^{2+}\), store depletion, and subsequent activation of SOC channels lead to an accumulation of intracellular Na\(^+\) that can drive Ca\(^{2+}\) influx via the reverse mode of the NCX.

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Tissue preparation. All experimental procedures were approved by the McMaster University Animal Care Committee and conform to the guidelines set out by the Canadian Council on Animal Care. Tracheas were obtained from cows (136–454 kg) euthanized at the local abattoir and transported to the laboratory in ice-cold Krebs buffer (see Solutions and chemicals). When trachea were received in the laboratory, the epithelium was removed and tracheal ASM strips (~2–3 mm wide, ~10 mm long) were excised and used immediately or stored at 4°C for use up to 48 h.

Cell isolation. Tracheal ASM strips were digested in modified Hanks’ balanced salt solution (with NaHCO3, without CaCl2 and MgSO4) containing collagenase (Sigma blend type-F, 2 mg/ml) and elastase (type IV, 250 μg/ml). After a 30-min incubation period at 37°C, papain (30 μg/ml) and (-)-1,4-dithio-L-threitol (750 μg/ml) were added and the tissues were incubated for an additional 20–30 min. Cells were gently triturated with a wide-bore pipette and then centrifuged to form a loose pellet. Supernatant was removed, and cells were resuspended in standard Ringer solution (see Solutions and chemicals). Phase-dense cells that exhibited contractile responses to caffeine (10 mM) were used for fluorimetric studies.

Organ bath studies. Intact segments of tracheal ASM were mounted in 4-ml organ baths with the use of silk thread (Ethicon 4-0) such that one end of the tissue was anchored and the other fastened to a Grass FT.03 force transducer, and preload tension of 1.0–1.5 g was applied. Isometric tension was digitized at 2 Hz and recorded online using the DigiMed System Integrator program (MicroMed, Louisville, KY). Tissues were bathed in modified Krebs buffer bubbled with 95% O2-5% CO2 and heated to 37°C. During a 1-h equilibration, tissues were repeatedly washed with modified Krebs buffer. To test for tissue responsiveness and viability, ASM strips were challenged with 60 mM KCl. The KCl was then washed out, and tissues were allowed to recover before experiments were conducted. Successive ACh-, 5-HT-, and histamine-induced contractions were triggered by EC50 concentrations for the bovine tracheal ASM (3 × 10−7 M ACh, 1.5 × 10−6 M 5-HT, and 5 × 10−6 M histamine), whereas caffeine-induced contractions were triggered by a concentration described in previous reports (14).

Ca2+ fluorimetry studies. Isolated tracheal ASM cells (see Cell isolation) were incubated with fluo-4 AM (2 μM, containing 0.1% Pluronic F-127) for 30 min at 37°C. Cells were then placed in a Plexiglas recording chamber and superfused with Ringer solution for a period of 30 min before experimentation to allow for complete dye deesterification. KB-R7943 was delivered via the bathing solution, whereas caffeine was delivered via a micropipette (Picospritzer II; General Valve, Fairfield, NJ). Confocal microscopy was performed at room temperature (21–23°C) with a custom-built apparatus (32) based on an inverted Nikon Eclipse TE2000-4 microscope using a ×40 S Fluor oil objective. Briefly, 488-nm illumination from a photodiode laser was scanned across an isolated cell in X and Y planes by using two mirrors oscillating at 8 kHz and 30 Hz, respectively. The emitted fluorescence (>500 nm) was detected using a photomultiplier; the signal was then digitized, and images were generated (1 frame/s, 480 × 400 pixels) that were stored in TIFF stacks of several hundred frames on a local hard drive using image-acquisition software (Video Savant 4.0; IO Industries, London, ON, Canada). Image files were then imported into Scion (free download: www.scioncorp.com) for subsequent analysis, using a custom-written macro designed to deter-
Fig. 3. Representative tracings of ACh (3 × 10⁻⁷ M; filled boxes)-induced contractions of bovine ASM are shown in the absence of Na⁺ and the presence of ouabain (10⁻⁶ M; A) and in the presence of ouabain (10⁻⁶ M) alone (B). Mean changes in peak magnitudes of ACh-induced contractions are shown in the absence of extracellular Na⁺ and the presence of ouabain (10⁻⁶ M; C) and in the presence of ouabain (10⁻⁶ M) alone (D), each compared with control responses. Mean changes in baseline force are shown in the absence of extracellular Na⁺ and the presence of ouabain (10⁻⁶ M; E) and in the presence of ouabain (10⁻⁶ M) alone (F), each compared with control responses. W, response following reintroduction of extracellular Na⁺ and/or washing with clean buffer. Changes in ACh-induced contractions are expressed as percent change from the first ACh-evoked response. Changes in baseline force are expressed as a percentage of the 60 mM KCl response. *P < 0.05 compared with control responses, n = 4–6.
mine average fluorescence intensity over a defined nonnuclear region of interest.

Solutions and chemicals. Modified Krebs buffer used in organ bath studies consisted of (in mM) 116 NaCl, 4.6 KCl, 1.2 MgSO4, 2.5 CaCl2, 1.3 NaH2PO4, 23 NaHCO3, 11 d-glucose, 0.01 indomethacin, 0.0001 propranolol, and 0.1 Nω-nitro-L-arginine (L-NNA) bubbled with 95% O2-5% CO2 to maintain pH 7.4. Zero-Na+ Krebs buffer consisted of (in mM) 116 N-methyl-D-glucamine, 4.6 KCl, 1.2 MgSO4, 2.5 CaCl2, 1.3 KH2PO4, 23.0 KHCO3, 11 d-glucose, 0.01 indomethacin, 0.0001 propranolol, and 0.1 L-NNA set to a pH of 7.4 with 5 N HCl and continuously bubbled with 95% O2-5% CO2 to maintain pH at 7.4. Dissociation buffer consisted of Ca2+ and effect on baseline tone summarized (Fig. 3C and effect on baseline tone summarized in Fig. 3E).

To confirm the findings of the aforementioned Na+ depletion studies, we employed the same repetitive contraction protocol in tissues pretreated with KB-R7943 (2 × 10⁻⁵ M), a selective inhibitor of the reverse mode of the NCX (i.e., Ca2+ influx mode) (3, 8, 15, 35, 37). Selective inhibition of the reverse mode of the NCX markedly reduced successive ACh-induced contractions (Fig. 4A; summarized in Fig. 4B) without altering the resting tone between responses (Fig. 4A; summarized in Fig. 4C). Although these effects were similar to those observed upon removal of extracellular Na+, the KB-R7943-induced contractions of bovine ASM in the presence of KB-R7943 (2 × 10⁻⁵ M).
induced effect occurred later into the series of successive ACh-induced contractions.

Effect of inhibition of the NCX on successive histamine-induced contractions. Reproducible histamine-induced contractions of bovine tracheal ASM could be triggered in 15-min intervals (Fig. 5A). Removal of extracellular Na\(^+\) led to a reversible decrease in the peak magnitude of successive histamine-induced contractions and an increase in resting tone (Fig. 5B; summarized in Fig. 5D). As observed in Fig. 5B, reintroduction of Na\(^+\) caused a transient contraction followed by a relaxation of the tissues to baseline tone. Inhibition of Ca\(^{2+}\) influx via the reverse mode of NCX by KB-R7943 (2 × 10\(^{-5}\) M) led to a smaller, but statistically significant, reduction in histamine-induced contractions (Fig. 5C; summarized in Fig. 5E).

Effect of inhibition of the NCX on successive 5-HT-induced contractions. As observed with ACh and histamine, reproducible 5-HT-induced contractions could be triggered in 15-min intervals (Fig. 6A). Removal of extracellular Na\(^+\) reversibly attenuated 5-HT-induced contractions (Fig. 6B; summarized in Fig. 6D). Furthermore, zero-Na\(^+\) conditions led to a gradual elevation in resting tone evidenced by an incomplete relaxation to previous baseline tone before addition of contractile agent (Fig. 6B). Addition of KB-R7943 (2 × 10\(^{-5}\) M) led to a delayed, but statistically significant, reduction in successive 5-HT-induced contractions (Fig. 6C; summarized in Fig. 6E).

Effect of inhibition of the NCX on successive caffeine-induced contractions. To further examine the role of the reverse mode of the NCX in SR refilling, we triggered Ca\(^{2+}\) mobilization and contractions independent of plasmalemmal

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**Fig. 5.** Representative tracings of histamine (5 × 10\(^{-6}\) M; filled boxes)-induced contractions of bovine ASM are shown under control conditions (A), in the absence of Na\(^+\) (B), and in the presence of KB-R7943 (2 × 10\(^{-5}\) M; C). Mean changes in peak magnitudes of histamine-induced contractions are shown in the absence of Na\(^+\) (D) and in the presence of KB-R7943 (2 × 10\(^{-5}\) M; E). W, response following reintroduction of Na\(^+\) and/or washing with clean buffer. All mean values are expressed as percent change from the first histamine-evoked response. *P < 0.05; **P < 0.005 compared with control responses, n = 3-5.
receptor activation. To accomplish this, ASM strips were challenged with caffeine (10 mM), an agent that triggers Ca\textsuperscript{2+}/H\textsuperscript{11001}/release from the SR via activation of ryanodine receptors. As with the receptor-mediated responses, consistent caffeine-induced contractions could be evoked in 15-min intervals (Fig. 7A), although these were much smaller in magnitude than the latter (likely due to the absence of a contribution from Ca\textsuperscript{2+}-sensitization pathways). Removal of extracellular Na\textsuperscript{+} led to an elevation in the baseline tone and a significant reduction of caffeine-induced contractions (Fig. 7B; summarized in Fig. 7D). Furthermore, the introduction of KB-R7943 (2 × 10\textsuperscript{-5} M) led to significant reduction in caffeine-induced contractions, an effect that did not display reversibility (Fig. 7C; summarized in Fig. 7E).

**Effect of zero Na\textsuperscript{+} and KB-R7943 on KCl-induced contractions.** KCl is widely used to trigger voltage-dependent Ca\textsuperscript{2+}-influx (22, 23, 26, 31, 39) and has also recently been shown to stimulate the RhoA/ROCK signaling pathway (9, 18, 25, 30, 36), but it does not evoke Ca\textsuperscript{2+} release in ASM. To determine whether our interventions targeting the NCX activity had any effect on those signaling events outside of Ca\textsuperscript{2+} release, we examined contractions triggered by KCl (60 mM). Selective inhibition of L-type Ca\textsuperscript{2+} channels with nifedipine (10\textsuperscript{-5} M) nearly abolished KCl-induced contractions (Fig. 8B; summar...
rized in Fig. 8E). In contrast, selective inhibition of the reverse mode of the NCX with KB-R7943 (2 × 10⁻⁵ M; Fig. 8C; summarized in Fig. 8D) and removal of extracellular Na⁺ (Fig. 8B; summarized in Fig. 8E) had no significant effects on KCl-induced contractions.

**Effect of inhibition of the reverse mode of the NCX on successive caffeine-induced Ca²⁺ transients in single ASM cells.** To determine whether the attenuations observed in our contractile experiments were due to altered Ca²⁺ handling, and more specifically, to reduced store refilling, we used Ca²⁺ fluorimetry to examine the effects of KB-R7943 on changes in [Ca²⁺]. Consistent caffeine-induced (10 mM) increases in [Ca²⁺] could be triggered in 5-min intervals. In agreement with our contractile data, selective inhibition of the reverse mode of the NCX with KB-R7943 (2 × 10⁻⁵ M) nearly abolished caffeine-induced increases in [Ca²⁺], (Fig. 9A; summarized in Fig. 9B). Interestingly, the introduction of KB-R7943 led to a small decrease in resting [Ca²⁺]; in some cells (Fig. 9A), but this was not statistically significant when the data were pooled.

**DISCUSSION**

A novel role for the reverse mode of the NCX in ASM is supported by our current study suggesting that Ca²⁺ influx via the reverse mode of the NCX provides a source of Ca²⁺ for refilling of internal stores (i.e., SR) following agonist stimulation. Refilling of the SR following agonist-induced Ca²⁺ release is an integral part of excitation-contraction coupling in ASM. Elevations in [Ca²⁺], that trigger contraction of ASM are heavily dependent on agonist-induced generation of second messengers and subsequent release of Ca²⁺ from the SR. The extracellular domain provides an infinite source of Ca²⁺ for refilling of internal Ca²⁺ stores. Calcium can enter smooth muscle cells via activation of L-type Ca²⁺, ROC, and SOC channels.

Our data suggest that the reverse mode of the NCX plays a substantial role in the refilling of the SR following agonist stimulation, whereas others have reported the involvement of L-type Ca²⁺ channels (5–7, 11). However, these studies used depolarizing stimuli (i.e., KCl-induced depolarization) to trigger the refilling process, which also favors the reverse mode of
Furthermore, Bourreau et al. (7) reported that nifedipine, an inhibitor of L-type Ca\(^{2+}\) channels, only reduced repetitive ACh-induced contractions by one-third over a course of nine contractions, suggesting the existence of additional Ca\(^{2+}\) influx pathways that contribute to SR refilling.

Calcium influx pathways involving the NCX have been described in vascular (1, 40), urethral (8), and airway smooth muscle (10). The NCX is a bidirectional transporter that normally catalyzes the movement of 3 Na\(^+\)/H\(^+\) into a cell in exchange for the extrusion of 1 Ca\(^{2+}\)/H\(^+\). However, the transmembrane Na\(^+\)/H\(^+\) gradient (resulting from an influx and elevation of intracellular Na\(^+\)) and membrane potential (cell depolarization) can be harnessed to push the exchanger in the reverse mode, triggering the efflux of Na\(^+\) and subsequent influx of Ca\(^{2+}\) (4). The theoretical reversal potential of the NCX (approximately −35 mV), reported by Eisner and Lederer (12) suggests that the forward mode (i.e., Ca\(^{2+}\) extrusion mode) of the NCX is favored under resting conditions, whereas membrane depolarization during excitation (greater than −30 mV) can trigger Ca\(^{2+}\) entry via the reverse mode. We (14) recently reported that mobilization of Ca\(^{2+}\) triggered by cholinergic stimulation or caffeine treatment leads to membrane depolarization into the range necessary to induce Ca\(^{2+}\) entry via the reverse mode of the NCX.

Agonist-induced activation of G protein-coupled receptors results in the mobilization of Ca\(^{2+}\). The depletion of the SR increases the open probability of SOC channels, thought to be composed of TRP proteins, that constitute an inward “store-operated” cation conductance (13). These channels are permeable to both Na\(^+\) and Ca\(^{2+}\) and are thought to contribute to refilling of the SR via capacitative Ca\(^{2+}\) entry (27).

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**Fig. 8.** Representative tracings of KCl (60 mM; filled boxes)-induced contractions of bovine ASM are shown under control conditions (A), in the presence of nifedipine (10 \(-5\) M; B), in the presence of KB-R7943 (2 \(\times\) \(10^{-5}\) M; C), and in the absence of Na\(^+\) (D). Mean changes in peak magnitude of KCl-induced contractions are shown under control conditions, in the presence of nifedipine (10 \(-5\) M), in the presence of KB-R7943 (2 \(\times\) \(10^{-5}\) M), and in the absence of Na\(^+\) (E). W, response following reintroduction of Na\(^+\) and/or washing with clean buffer. All mean values are expressed as percent change from the first KCl-evoked response. **P < 0.005 compared with all conditions, n = 4–5.
Agonist stimulation and the resulting activation of SOC channels can lead to an influx of Na\(^+\) from the extracellular domain (13). The increase in intracellular Na\(^+\) in conjunction with the activation of depolarizing membrane currents (i.e., Ca\(^{2+}\)-dependent Cl\(^-\) currents) provide the conditions necessary for Ca\(^{2+}\) influx via the reverse mode of the NCX. Indeed, ASM displays cation conductances that reflect SOC activity and Cl\(^-\) channels that contribute to increases in intracellular Na\(^+\) and membrane depolarization, respectively (13, 14, 16, 17). Furthermore, the existence of the NCX in ASM is well established (2, 20), and its functional existence in excitation-contraction has been reported recently (10). Thus it is plausible that Na\(^+\) influx following agonist-induced store depletion, in conjunction with membrane depolarization, could drive Ca\(^{2+}\) influx through the reverse mode of the NCX.

In the present work we have employed two distinct approaches to alter the activity of the NCX to examine its role in providing a conduit for Ca\(^{2+}\) influx contributing to the refilling of the SR. Removal of extracellular Na\(^+\) causes a transient increase in the reverse mode activity, followed by an inhibition of both forward (i.e., Ca\(^{2+}\) extrusion) and reverse (i.e., Ca\(^{2+}\) influx) modes of the NCX (8, 40). Successive agonist-induced contractions (ACh, histamine, 5-HT) of ASM were significantly reduced in the absence of extracellular Na\(^+\), suggesting a loss of Ca\(^{2+}\) influx required for adequate refilling of the SR.

This effect was unchanged in the presence of ouabain; thus the reductions in successive responses during Na\(^+\) depletion do not involve the Na\(^+\)/K\(^+\) pump. However, ouabain alone had a biphasic effect on agonist-induced contractions, potentiating the early responses (responses 1–5) and attenuating the last two responses (Fig. 3B; summarized in Fig. 3D). Whereas removal of Na\(^+\) will inhibit both the Na\(^+\)/K\(^+\) pump and the NCX, ouabain only inhibits the former. In fact, by depolarizing the membrane and allowing Na\(^+\) to accumulate under the plasma-lemma, ouabain will actually enhance reverse-mode NCX, thereby raising baseline tone and augmenting agonist-evoked contractions. The subsequent suppression of these mechanical responses much later in the protocol could be due to various other ionic disruptions caused by prolonged accumulation of Na\(^+\) and/or Ca\(^{2+}\), or loss of cytosolic K\(^+\), including acidosis or osmotic effects. In contrast to contractions evoked by receptor-dependent mechanisms, removal of extracellular Na\(^+\) had no effect on KCl-induced contractions (i.e., depolarization-induced contractions), suggesting that the reduction in successive agonist-induced contractions is not due to an inhibition of Ca\(^{2+}\) influx through L-type Ca\(^{2+}\) channels.

The second approach used to elucidate the role of the NCX in store refilling was to treat tissues with KB-R7943, a selective inhibitor of the reverse mode of the NCX (3, 8, 15, 35, 37). Studies examining the pharmacology of KB-R7943 in cardiac myocytes report it suppresses the reverse mode 60 times more strongly than the forward mode (37). In the context of Ca\(^{2+}\) handling, Bradley et al. (8) reported that KB-R7943 had no effect on Ca\(^{2+}\) release from ryanodine and inositol 1,4,5-trisphosphate-sensitive stores or the inward current mediated by Ca\(^{2+}\)-dependent Cl\(^-\) channels in rabbit urethra smooth muscle. Dai et al. (10) have reported that KB-R7943, at the concentration used in our study, abolished ACh-induced asynchronous Ca\(^{2+}\) oscillations in intact porcine tracheal ASM, an effect attributed to inhibition of the reverse mode of the NCX. Furthermore, Kawano et al. (19) reported that high concentrations of KB-R7943 (30–100 μM) reduced spontaneous Ca\(^{2+}\) oscillations in human mesenchymal stem cells but had no effect on the lanthionum-sensitive component of these responses mediated by nonselective cation channels. Introduction of KB-R7943 in our experiments led to a significant decrease in successive contractions induced by the activation of plasmalemmal receptors (ACh, histamine, 5-HT) and direct release of Ca\(^{2+}\) from the SR via activation of ryanodine receptors (caffeine). As in the Na\(^+\) depletion experiments, introduction of KB-R7943 had no effect on Ca\(^{2+}\) influx via L-type Ca\(^{2+}\) channels (i.e., had no effect on KCl-induced contractions). Furthermore, KB-R7943 nearly abolished successive caffeine-induced increases in [Ca\(^{2+}\)], measured by Ca\(^{2+}\) fluorimetry.

Together, our data suggest that Ca\(^{2+}\) influx through the reverse mode of the NCX contributes substantially to increasing the pool of intracellular Ca\(^{2+}\) necessary for refilling of the SR. Since the removal of extracellular Na\(^+\) and KB-R7943 had no effect on contractions mediated by KCl [involving L-type Ca\(^{2+}\) channels (22, 23, 26, 31, 39) and RhoA/ROCK signaling (9, 18, 25, 30, 36)] but significantly reduced agonist-induced contractions (involving those same pathways as well as release of the internal Ca\(^{2+}\) pool), we conclude that the reductions observed during our interventions were due to specific targeting of the reverse mode of the NCX and subsequent refilling of...
the internal Ca\(^{2+}\) pool. Thus the data are consistent with our hypothesis that Ca\(^{2+}\)-influx via the reverse mode of the NCX is essential to the refilling of the SR following Ca\(^{2+}\) mobilization by a variety of physiological agonists.

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