Hypoxic pulmonary vasoconstriction in intact rat intrapulmonary arteries is not initiated by inhibition of Na\(^+\)-Ca\(^{2+}\) exchange

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Submitted 14 September 2006; accepted in final form 3 July 2007

Becker S, Moir LM, Snetkov VA, Aaronson PI. Hypoxic pulmonary vasoconstriction in intact rat intrapulmonary arteries is not initiated by inhibition of Na\(^+\)-Ca\(^{2+}\) exchange. Am J Physiol Lung Cell Mol Physiol 293: L982–L990, 2007. First published July 6, 2007; doi:10.1152/ajplung.00361.2006.—It has been proposed that a hypoxia-induced inhibition of the Na\(^+\)-Ca\(^{2+}\) exchanger (NCX) contributes to hypoxic pulmonary vasoconstriction (HPV). By recording isometric tension development in rat intrapulmonary arteries (IPA), we examined the effect on HPV of maneuvers that reduce the ability of NCX to regulate intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\text{i}). In some experiments, fura pentakis(acetoxymethyl) ester-3 (fura PE-3) was also used to monitor [Ca\(^{2+}\)]\text{i}. HPV was elicited in IPA that were pretreated with 10 \(\mu\)M diltiazem and slightly preconstricted with PGF\(_2\alpha\), which enhances the hypoxic response. Substitution of Na\(^+\) with Li\(^+\) increased HPV and the associated rise in [Ca\(^{2+}\)]\text{i}. Pretreatment with ouabain (100 \(\mu\)M) to diminish the Na\(^+\) gradient or with the reverse-mode NCX inhibitor KB-R7943 (3 or 10 \(\mu\)M) had no significant effect on HPV. Combined treatment with ouabain and low-[Na\(^+\)] (24 mM) solution enhanced HPV strongly. The role of NCX in HPV in Ca\(^{2+}\) extrusion was examined by assessing the decrease in [Ca\(^{2+}\)]\text{i} in Ca\(^{2+}\)-free physiological saline solution either containing or lacking Na\(^+\) following a high K\(^+\)-induced loading of cellular [Ca\(^{2+}\)]. Although the large initial rapid fall in [Ca\(^{2+}\)]\text{i} was Na\(^+\) independent, final recovery of [Ca\(^{2+}\)]\text{i} to its basal level was delayed in the absence of Na\(^+\). Therefore, HPV persisted or was increased under conditions in which forward-mode NCX was already attenuated or prevented, demonstrating that inhibition of NCX by hypoxia is unlikely to initiate HPV. Instead, NCX appears to act to inhibit HPV as would be expected if it is functioning to extrude Ca\(^{2+}\).
KB-R7943, affected HPV in isolated intrapulmonary arteries (IPA) of the rat. Our results suggest that NCX does not contribute importantly to the effects of hypoxia on contraction in this preparation.

METHODS

Tension measurements. Adult male Wistar rats (250–275 g) were killed by cervical dislocation in accordance with Schedule 1 as prescribed and approved by the UK Home Office and the Guide for the Care and Use Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85-23, revised 1996). The heart and lungs were rapidly removed and placed into cold Krebs physiological saline solution (PSS; in mM: 118 NaCl, 24 NaHCO3, 1 MOPS, 0.44 NaH2PO4, 4 KCl, 5.5 glucose, and 1.8 CaCl2). Small exposures to hypoxia. Aarhus) were loaded with the Ca2+

METHODS

In most experiments, the artery was first exposed to hypoxia over baseline recorded at the beginning of experiments during a 2-min period of small PA to hypoxia (9). Arteries were then exposed to a hypoxic gas mixture (5% CO2, balance nitrogen) for 40 min. Vessels were allowed to recover in PSS under normoxic conditions for at least 1 h between successive exposures to hypoxia. All experiments were conducted in the presence of diltiazem (ouabain, KB-R7943) or a solution change (Na+ substitution in IPA preconstricted to 80 mM K+; [Na+]i, arteries were separated by at least an hour, were carried out (n = 4; data not shown). For measurement of pH, arteries were loaded with 10 μM BCECF/AM for 1 h at 37°C. After removal of the dye, arteries were illuminated alternatively at 490 nm and 440 nm, and the ratio of emission intensities (R490/440) was recorded with parallel tension. At the end of the experiment, arteries were incubated in a series of solutions containing 140 mM KC1, 10 mM HEPES, 1 mM EGTA, and 2 μg/ml nigericin, in which the pH had been adjusted with 0.5 M KOH to values of between 6 and 8. Steady-state values of R490/440 were plotted against pH, and the resulting mean data were fitted with a Hill curve that was used to convert R490/440 values recorded during the experiment to pHi.

Statistical analysis. Tension and ΔF340/380 were measured just before hypoxia was imposed, at the peak of the phase 1 HPV contraction, and thereafter at 5-min intervals throughout HPV and reoxygenation. Results in Figs. 3–7 are presented as means ± SE of these measurements. Student’s t-test for paired or unpaired data was performed, as appropriate, to evaluate statistical significance. Differences were assumed to reach statistical significance at P < 0.05 and are indicated by asterisks.

Chemical solutions. NaCl, KCl, LiCl, glucose, and CaCl2 were obtained from BDH; MOPS, choline bicitarate, diltiazem, ouabain, acetylcholine, l-NAMe, and fura PE-3/AM were from Sigma; NaHCO3 was from Fisher Scientific; prostaglandin E2, tromethamine (PFG2) was from Biomol; KB-R7943 was from Tocris, and gases were from BOC. In all experiments, the concentration of PGF2α was 10 μM to eliminate effects caused by changes in membrane potential. Drugs were added to the solution at least 15 min before the imposition of hypoxia.

Tension development during hypoxia was expressed as a percentage of the maximal contraction, measured as the increase in tension over baseline recorded at the beginning of experiments during a 2-min exposure of the artery to 80 mM K+. This “pretone” has previously been described to greatly enhance the contractile response of small PA to hypoxia (9). Arteries were then exposed to a hypoxic gas mixture (5% CO2, balance nitrogen or 1% O2, 5% CO2, balance nitrogen) for 40 min. Vessels were allowed to recover in PSS under normoxic conditions for at least 1 h between successive exposures to hypoxia. All experiments were conducted in the presence of diltiazem (10 μM) to eliminate effects caused by changes in membrane potential. Drugs were added to the solution at least 15 min before the imposition of hypoxia.

Ca2+ and pH measurements. For measurement of [Ca2+], arteries mounted on a confocal wire myograph (Danish Myo Technology, Aarhus) were loaded with the Ca2+-sensitive fluorescent dye fura PE-3/AM (4 μM) for 1 h at 37°C. The dye was flushed from the solution, and the myograph was then transferred onto the stage of an inverted microscope (Nikon Diaphot TMD 200). Vessels were illuminated alternately at 340 and 380 nm using Optonac monochromator (Cairn Research), and fluorescence at >510 nm was recorded parallel with tension using Acquisition Engine software (Cairn Research). Data were collected and stored on a PC for offline analysis. Changes in [Ca2+]i during HPV are shown as the effect of hypoxia on the F340/F380 Ratio (ΔF340/F380) normalized to the effect of 80 mM K+ on this ratio. Time control experiments showed that ΔF340/F380 was not significantly different when two 40-min exposures to hypoxia, separated by at least an hour, were carried out (n = 4; data not shown).

For measurement of pH, arteries were loaded with 10 μM BCECF/AM for 1 h at 37°C. After removal of the dye, arteries were pH-metered with a glass electrode.
Application of NMDG-PSS in the presence of L-NAME caused a transient contraction in PGF2α-preconstricted pulmonary arteries (IPA) that was similar in magnitude to that caused by Li⁺-PSS. However, this was not followed by the progressive development of a slow contraction, so that within 15 min, tension had returned to the level observed in normal PSS (Fig. 1A, open triangles in Fig. 1B). Thus, in this case, abrogation of NCX function did not produce a contraction that mimicked HPV.

A second prediction of the hypothesis that HPV can be largely explained by a hypoxia-induced suppression of NCX activity (25) is that hypoxia should have little or no effect on contraction if NCX is already inhibited. We next examined whether this was the case. When arteries were preconstricted with PGF2α and then exposed to Li⁺-PSS in the continued presence of agonist, subsequent hypoxic challenge consistently evoked HPV (n = 4; Fig. 2). Under these conditions, contractions in response to hypoxia were similar in size or larger than in normal [Na⁺] PSS, and underlying constriction was greatly enhanced. However, because application of Li⁺-PSS tended to raise the pretone level, which could have influenced the response to hypoxia (14), in subsequent experiments arteries were preincubated for at least 15 min with Li⁺-PSS (or under other conditions designed to inhibit NCX) before preconstriction with PGF2α, the concentration of which was then adjusted so that the pretone level closely matched that imposed during the control response.

Using this approach, pretreatment with Li⁺-PSS did not significantly affect the contractile response during phase 1 of HPV. However, tension after phase 1, which normally fell sharply within 5 min, remained markedly elevated. Contraction after 40 min of hypoxia (phase 2) was also substantially increased. However, since the underlying pretone contraction was also enhanced, the fall in tension upon reoxygenation was not significantly different than in normal [Na⁺] PSS (Figs. 3A and 8). Similar results were obtained in two experiments in which diltiazem was not included in the solution (not shown).

Measurement of [Ca^{2+}]_i in similar experiments (Fig. 3B) indicated that the rise in [Ca^{2+}]_i during phase 1 was unaffected by Na⁺ substitution. However, the subsequent decline in [Ca^{2+}]_i, which was prominent under control conditions, was
LG IN INTACT RAT IPA IS NOT INITIATED BY INHIBITION OF Na\(^+\)-Ca\(^{2+}\) EXCHANGE

Fig. 2. Na\(^+\)-free solution ([Na\(^+\])\(_e\) = 0.44 mM) does not prevent hypoxic pulmonary vasconstriction (HPV) in isolated rat small pulmonary arteries. Representative trace shows force development to hypoxia in a PGF\(_{2\alpha}\)-preconstricted IPA in the presence of extracellular Na\(^+\) (left) and after substitution of extracellular Na\(^+\) (Li\(^+\)-PSS; [Na\(^+\])\(_e\) = 0.44 mM; right).

significantly slowed in the absence of Na\(^+\), although ultimately [Ca\(^{2+}\)]\(_i\) fell to a level that was not significantly different from that observed under control conditions.

When Na\(^+\) was substituted with NMDG using the same protocol, phase 1 HPV was significantly attenuated, whereas phase 2 was increased to an extent similar to that seen in Li\(^+\)-PSS (Fig. 4). In most experiments, arteries failed to relax following reoxygenation, and in some cases, tension even continued to increase, an effect that was not apparent when Li\(^+\)-PSS was used.

KB-R7943 is used as a selective inhibitor of reverse-mode NCX (e.g., Ref. 5) and has been shown to prevent the rise in [Ca\(^{2+}\)] in arterial myocytes caused by Na\(^+\) removal with an IC\(_{50}\) of 3.5 μM (22). At concentrations of 3 and 10 μM (Fig. 5), KB-R7943 did not alter either the phase 1 or phase 2 contractions. The fall in tension upon reoxygenation also remained unaltered (Figs. 5 and 8). Ouabain (100 μM), which increases [Na\(^+\)], thus promoting Ca\(^{2+}\) influx and inhibiting Ca\(^{2+}\) extrusion by NCX, also had no significant effects on either phase 1 or phase 2, or on the fall in tension upon reoxygenation (Figs. 6 and 8).

Finally, pretreatment with the combination of partial substitution of Na\(^+\) by Li\(^+\) ([Na\(^+\])\(_e\) = 24 mM) and ouabain (100 μM) caused a large increase of phase 1 and 2 and also in the underlying pretone after HPV. The increase in phase 2 was significantly greater than that for the pretone contraction, showing that phase 2 itself was unambiguously enhanced under these conditions (Figs. 7 and 8).

To assess the extent to which NCX contributes to Ca\(^{2+}\) extrusion in IPA, the recovery of [Ca\(^{2+}\)] following the loading of Ca\(^{2+}\) into cells was assessed in fura PE-3-loaded arteries. IPA were incubated in nominally Ca\(^{2+}\)-free PSS and treated with either thapsigargin (Thg; 1 μM, n = 4) or cyclopiazonic acid (CPA, 30 μM, n = 3) to prevent Ca\(^{2+}\) uptake into the sarcoplasmic reticulum. IPA were then exposed to PSS containing 0.44 mM Na\(^+\), 1.8 mM Ca\(^{2+}\), and 30 mM K\(^+\) to depolarize the smooth muscle cells and load them with Ca\(^{2+}\) via L-type Ca\(^{2+}\) channels. After 10 min, IPA were then placed into Ca\(^{2+}\)-free PSS containing either normal or 0.44 mM [Na\(^+\)]. Representative traces of the resulting changes in [Ca\(^{2+}\)] were shown for IPA treated with CPA (Fig. 9A) and Thg (Fig. 9B). Since Thg and CPA work similarly, and since the results obtained with these two drugs were indistinguishable, the data obtained using both drugs were combined and are shown in Fig. 9C. Figure 9 illustrates that although the initial rapid fall in [Ca\(^{2+}\)], that occurred in Ca\(^{2+}\)-free solution when the depolarizing stimulus was terminated was little affected by the absence of a Na\(^+\) gradient, the final recovery of [Ca\(^{2+}\)] to a basal level was significantly delayed in the low-[Na\(^+\)] PSS. As shown in Fig. 9B, if normal [Na\(^+\)] was restored during the...
Hypoxia decreased HPV in experiments carried out using the same protocol as for Fig. 3. Values are means ± SE, n = 11 for control (open circles) and 9 for NMDG (closed circles). Asterisks represent time points at which HPV was significantly different than control, whereas † indicates that the PGE2-induced contraction was increased compared with control.

Delayed recovery phase in the continuing absence of Ca^{2+}, [Ca^{2+}]_i, then fell rapidly. These data are consistent with the idea that NCX makes a small but significant contribution to the extrusion of Ca^{2+} from rat smooth muscle cells.

Figure 10 depicts the effect of Li^+-PSS, NMDG-PSS, and 24 mM Na^+-PSS + 100 µM ouabain on pH_i. The baseline pH_i measured using BCECF was 7.33 ± 0.04 (n = 9). Na^+ substitution caused a rapid and transient cellular alkalinization followed by a small steady-state acidification. The steady state ΔpH_i values were -0.048 ± 0.022 (n = 7), -0.078 ± 0.017 (n = 9), and -0.083 ± 0.027 (n = 8) in Li^+-PSS, NMDG-PSS, and 24 mM Na^+ + 100 µM ouabain, respectively.

**DISCUSSION**

NCX has previously been implicated in the removal of intracellular Ca^{2+} from smooth muscle, especially during smooth muscle cell stimulation when [Ca^{2+}]_i, is increased (3). Using freshly isolated smooth muscle cells from rat small pulmonary arteries, Wang et al. (25) observed that both Na^+ removal and hypoxia similarly slowed recovery from a sudden Ca^{2+} load. Moreover, hypoxia had no additional effect once NCX had been inhibited by Na^+ removal. These experiments indicated that inhibition of NCX and therefore Ca^{2+} extrusion during hypoxia could play a key role in the accumulation of [Ca^{2+}]_i in pulmonary artery smooth muscle cells that occurs during HPV.

However, it is alternatively possible that the activation rather than inhibition of NCX could contribute to HPV. The opening of nonselective cation channels, either those associated with store emptying or directly activated by agonists acting on receptors, is known to cause Na^+ influx (2). NCX has been shown to be localized to the areas of the plasmalemma that are closely apposed to peripheral elements of the endoplasmic reticulum (12). It has therefore been proposed that rises in [Na^+]_i in the narrow junctional space between the endoplasmic reticulum, whether caused by agonists (10) or by inhibition of the α3-isofrom-based type of the Na^+-K^+-ATPase, which is also localized to these regions of the plasmalemma (6), act to reverse NCX, thus causing Ca^{2+} influx and loading the ER with Ca^{2+}. Since evidence has been presented that Ca^{2+} release and SOC are important in raising [Ca^{2+}]_i during HPV (24), and that these channels are mainly permeable to Na^+ in pulmonary artery smooth muscle cells (21), it is conceivable...
that NCX could serve as an important pathway for Ca\(^{2+}\) influx during HPV.

In this study, we sought to determine whether NCX might play either of these roles in a well-characterized model of HPV in isolated IPA (16, 17). If inhibition of NCX by hypoxia made an important contribution to the development of HPV, procedures designed to affect NCX would be expected to alter HPV along predictable lines. In particular, inhibition of the ability of NCX to mediate Ca\(^{2+}\) extrusion should both mimic HPV and prevent or attenuate subsequent effects of hypoxia on both [Ca\(^{2+}\)]\(_i\) and contraction.

We tested this prediction first by examining the effect of substituting Na\(^+\) on tension development in IPA that had been slightly preconstricted with PGF\(_{2\alpha}\), under which condition hypoxia causes a biphasic contraction (17). Substitution of Na\(^+\) by Li\(^+\) caused a transient relaxation followed by a slow contraction. Because there is evidence that hypoxia inhibits nitric oxide release (1), we repeated these experiments in endothelium-denuded arteries and in the presence of L-NAME to inhibit nitric oxide production and found that in both cases, Na\(^+\) substitution then caused a biphasic contraction similar to HPV. On the other hand, substitution of Na\(^+\) by NMDG had no significant effect on sustained contraction in PGF\(_{2\alpha}\)-preconstricted IPA, implying that it might have been the elevation in [Li\(^+\)] that was causing this contraction rather than Na\(^+\) substitution per se.

Na\(^+\) substitution with Li\(^+\) had no significant effect on phase 1 HPV. The effect of Li-PSS on phase 2 was more difficult to define precisely, because although the rise in tension after 40 min of hypoxia was significantly enhanced in Li-PSS, Fig. 1 indicates that the underlying contraction to PGF\(_{2\alpha}\) rose by about the same amount, providing an explanation of why the fall in tension upon reoxygenation remained unchanged. However, it is notable that this increase in pretone (Fig. 1) was too slow to explain the observation that tension immediately after phase 1 was greatly increased by Na\(^+\) substitution. Similarly, measurements of the hypoxia-induced rise in [Ca\(^{2+}\)]\(_i\) also indicated that although substitution of Na\(^+\) with Li\(^+\) did not significantly enhance the effect of prolonged hypoxia, it did appear to increase [Ca\(^{2+}\)]\(_i\) soon after phase 1. These results are consistent with the concept that NCX is contributing to the removal of Ca\(^{2+}\) from the cytoplasm during HPV, especially after it reaches its highest level during phase 1, but that other Ca\(^{2+}\)-removing mechanisms are also involved and are able eventually to compensate for its absence.

On the other hand, substituting Na\(^+\) with NMDG caused a marked attenuation of phase 1 HPV, although significantly increasing tension development during phase 2. Following a transient enhancement, NMDG did not affect the contraction caused by PGF\(_{2\alpha}\) (Fig. 1), implying that its effect on the sustained response to hypoxia reflected a genuine enhancement of phase 2 HPV.

The results observed in experiments utilizing both Na\(^+\) substitutes demonstrate that phase 2 HPV was not inhibited in the absence of a Na\(^+\) gradient, under which condition NCX is unable to mediate either the net entry or extrusion of Ca\(^{2+}\). It therefore seems that inhibition of NCX does not play an important role in sustained HPV in isolated IPA.

The results of these experiments are less easily interpreted with regard to the role of NCX in phase 1, since Na\(^+\) substitution with NMDG inhibited phase 1, whereas its replacement by Li\(^+\) had no effect. Although it is possible that the inhibition of phase 1 seen in NMDG is the “true” effect on this phase of inhibiting NCX, and that the lack of effect on phase 1 in Li\(^+\)-PSS is due to an enhancing effect of Li\(^+\) on contraction (as, for example, seen in Fig. 1) balancing out an attenuation caused by inhibition of NCX, it is also the case that neither ouabain nor KB-R7943 affected HPV (16). The concept that HPV is not initiated by a hypoxia-induced inhibition of NCX was also borne out by experiments utilizing ouabain and the combination of ouabain and partial Na\(^+\) substitution with Li\(^+\). Pretreatment with ouabain caused no significant effect on either HPV or the pretone contraction. On the other hand, combined pretreatment with ouabain and low (24 mM) Na\(^+\)-PSS, which in addition to inhibiting NCX-mediated Ca\(^{2+}\) extrusion would be expected to promote net Ca\(^{2+}\) influx via NCX more strongly than would ouabain alone (because extracellular Na\(^+\) competes with Ca\(^{2+}\) for entry via the exchanger; Ref. 3), increased both phases of HPV, particularly phase 2, thus suggesting that NCX is still active during HPV. Since HPV was either not inhibited or was increased as a result of these maneuvers, all of which would be expected to inhibit NCX-mediated Ca\(^{2+}\) extrusion, these results again do not support the concept that HPV is due primarily to inhibition of NCX-mediated Ca\(^{2+}\) extrusion.

As described above, recent reports have established that SOC-mediated Ca\(^{2+}\) entry is activated by hypoxia and have indicated that this pathway may be largely responsible for causing HPV (9, 24, 27). Our results do not, however, support...
the suggestion (26) that Na\(^+\) entry via SOC additionally promotes HPV by enhancing reverse-mode NCX. Substitution of extracellular Na\(^+\)/H\(^+\) by Li\(^+\)/H\(^+\) (which even if it entered the cell through cation channels could not be used by reverse-mode NCX to enhance Ca\(^{2+}\)/H\(^+\) entry), ouabain (by increasing resting [Na\(^+\)]\(_{i}\)), and KB-R7943 (by inhibiting reverse-mode NCX) would all be expected to interfere with this process, yet none of these maneuvers inhibited HPV.

Substitution of Na\(^+\) by either Li\(^+\) or NMDG caused a transient alkalinization followed by a small decrease in the steady state pHi. The transient initial alkalinization caused by Na\(^+\) removal was irrelevant with regard to HPV in these experiments because Na\(^+\) was always substituted at least 25 min before hypoxia was imposed. It also seems unlikely that the slight subsequent acidification could have been contributing to the generally facilitory effect of these solutions on HPV. For example, NMDG-PSS did not have any sustained effect on tension in PGF2\(_{12}\)/H\(_{9251}\)-preconstricted arteries, implying that the acidification per se does not increase tension even in preconstricted arteries. Moreover, Raffestin and McMurtry (15) showed that intracellular acidification caused a decrease in HPV in perfused rat lung, implying that if anything, the fall in pHi caused by Na\(^+\) removal might have attenuated the enhancement of HPV we recorded.

Fig. 9. Ca\(^{2+}\) extrusion in normal and low-[Na\(^+\)] solutions. A: fura PE-3-loaded IPA was incubated in Ca\(^{2+}\)-free PSS and exposed to 30 µM cyclopiazonic acid (CPA) to inhibit SERCA. The artery was then exposed to PSS containing 0.44 mM Na\(^+\), 1.8 mM Ca\(^{2+}\), and 30 mM K\(^+\) for 5 min to increase [Ca\(^{2+}\)]\(_{i}\), and subsequently returned to Ca\(^{2+}\)-free PSS to observe Ca\(^{2+}\) extrusion. Following a second Ca\(^{2+}\) loading, the artery was placed in Ca\(^{2+}\)-free PSS containing 0.44 mM Na\(^+\) (with Li\(^+\) as the Na\(^+\) substitute). B: an experiment similar to that shown in A, except that 1 µM thapsigargin (Thg) was used to inhibit SERCA, and recovery from Ca\(^{2+}\) loading was examined first in 0.44 mM Na\(^+\) and then in the normal Na\(^+\) concentration. C: results from 4 similar experiments using Thg and 3 experiments using CPA were combined by normalizing the data such that the baseline R\(_{50/50}\) was set to 0% and the R\(_{50/50}\) at the end of the Ca\(^{2+}\) loading period was set to 100%. Means ± SE values for data recorded at 1-min intervals are shown. *The two sets of points indicated by the bracket were significantly different (P < 0.01) as assessed by repeated measures ANOVA.

Fig. 10. Representative trace illustrating the effect of Na\(^+\) substitution on intracellular pH, which was measured using BCECF as described in METHODS.
Together, these results indicate that NCX does not make an appreciable contribution to the regulation of Ca\(^{2+}\) homeostasis in these cells, except perhaps when the Na\(^{+}\) gradient is removed or reversed and [Ca\(^{2+}\)], is sufficiently elevated. Thus, responses to hypoxia were enhanced in low-Na\(^{+}\) PSS, especially if ouabain was also present, but the smaller decrease in the Na\(^{+}\) gradient due to ouabain alone, or inhibition of NCX with KB-R7943, had no obvious effect on HPV. Moreover, complete substitution of Na\(^{+}\) with Li\(^{+}\) or NMDG did not affect basal tone, and even in the presence of PGF\(_{2\alpha}\) replacement of Na\(^{+}\) with NMDG did not further increase tension. In accordance with this concept, the absence of a Na\(^{+}\) gradient had only a minor effect on the recovery of [Ca\(^{2+}\)], following Ca\(^{2+}\) loading of the cells with a depolarizing solution. The rapid initial fall in [Ca\(^{2+}\)], during the recovery from Ca\(^{2+}\) loading was most likely due to the plasmalemmal Ca\(^{2+}\) ATPase, since IPA in these experiments were pretreated with Thg or CPA to inhibit SERCA. Presumably the plasmalemmal Ca\(^{2+}\) ATPase works in parallel with NCX to extrude Ca\(^{2+}\) from the cytoplasm, e.g., the mitochondria, may also exist and be more important than has heretofore been appreciated (22).

The results of Fig. 9 differ from those of Wang et al. (25), who showed that Na\(^{+}\) removal dramatically slowed the rate at which [Ca\(^{2+}\)], fell in isolated IPA myocytes following a Ca\(^{2+}\) load initiated by the application of caffeine and high-K\(^{+}\) solution. One possible explanation for these results is that we removed Ca\(^{2+}\) from the bath solution to measure Ca\(^{2+}\) extrusion, whereas they did not. Therefore, any increase in Ca\(^{2+}\) influx during the period when Ca\(^{2+}\) was falling (e.g., an enhancement of caffeine-stimulated SOC due to Na\(^{+}\) removal) could have been misinterpreted as an inhibition of extrusion. This might also have applied to their finding that hypoxia inhibited Ca\(^{2+}\) extrusion, since there is evidence that hypoxia not only releases Ca\(^{2+}\) to activate SOC but potentiates SOC when this has previously been activated by Ca\(^{2+}\) store depletion (24).

In summary, we have used Na\(^{+}\) substitution, Na\(^{+}\) pump inhibition, and the pharmacological NCX inhibitor KB-R7943 to assess the possible contribution of inhibition or reversal of NCX to HPV. Our results show that these procedures uniformly failed to inhibit HPV. Thus it seems unlikely that inhibition of NCX by hypoxia, or activation of its reverse mode due to hypoxia-induced Na\(^{+}\) entry, contributes significantly to HPV, at least in this model. Instead, NCX is probably functioning as one of several Ca\(^{2+}\)-removing pathways acting to limit hypoxia-induced increases in [Ca\(^{2+}\)], a role consistent with the enhancement of the PGF\(_{2\alpha}\)-induced contraction in IPA and with previous observations that it functions to promote Ca\(^{2+}\) extrusion in vascular smooth muscle under conditions in which the Na\(^{+}\) gradient is intact.

**REFERENCES**