KB-R7943 Block of Ca\(^{2+}\) Influx Via Na\(^{+}/\)Ca\(^{2+}\) Exchange Does Not Alter Twitches or Glycoside Inotropy but Prevents Ca\(^{2+}\) Overload in Rat Ventricular Myocytes

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**Background**—The Na\(^{+}/\)Ca\(^{2+}\) exchange (NCX) extrudes Ca\(^{2+}\) from cardiac myocytes, but it can also mediate Ca\(^{2+}\) influx, load the sarcoplasmic reticulum with Ca\(^{2+}\), and trigger Ca\(^{2+}\) release from the sarcoplasmic reticulum. In ischemia/reperfusion or digitalis toxicity, increased levels of intracellular [Na\(^{+}\)] ([Na\(^{+}\)]) may raise levels of intracellular [Ca\(^{2+}\)] ([Ca\(^{2+}\)]) via NCX, leading to cell injury and arrhythmia.

**Methods and Results**—We used KB-R7943 (KBR) to selectively block Ca\(^{2+}\) influx via NCX to study the role of NCX-mediated Ca\(^{2+}\) influx in intact rat ventricular myocytes. Removing extracellular Na\(^{+}\) caused [Ca\(^{2+}\)], to rise, due to Ca\(^{2+}\) influx via NCX, and this was blocked by 90% with 5 \(\mu\)mol/L KBR. However, KBR did not alter [Ca\(^{2+}\)], decline due to NCX. Thus, we used 5 \(\mu\)mol/L KBR to selectively block Ca\(^{2+}\) entry but not efflux via NCX. Under control conditions, 5 \(\mu\)mol/L KBR did not alter steady-state twitches, Ca\(^{2+}\) transients, Ca\(^{2+}\) load in the sarcoplasmic reticulum, or rest potentiation, but it did prolong the late low plateau of the rat action potential. When Na\(^{+}/K^{+}\) ATPase was inhibited by strophanthidin, KBR reduced diastolic [Ca\(^{2+}\)], and abolished the spontaneous Ca\(^{2+}\) oscillations, but it did not prevent inotropy.

**Conclusions**—In rat ventricular myocytes, Ca\(^{2+}\) influx via NCX is not important for normal excitation-contraction coupling. Furthermore, the inhibition of Ca\(^{2+}\) efflux alone (as [Na\(^{+}\)] rises) may be sufficient to cause glycoside inotropy. In contrast, Ca\(^{2+}\) overload and spontaneous activity at high [Na\(^{+}\)], was blocked by KBR, suggesting that net Ca\(^{2+}\) influx (not merely reduced efflux) via NCX is involved in potentially arrhythmogenic Ca\(^{2+}\) overload. (Circulation. 2000;101:1441-1446.)

**Key Words:** myocytes ■ ion exchange ■ sarcoplasmic reticulum ■ arrhythmia

Na\(^{+}/\)Ca\(^{2+}\) exchange (NCX) is the main mechanism of Ca\(^{2+}\) extrusion from cardiac myocytes, and 7% to 28% of cardiac relaxation is due to NCX. Less quantitative information is available concerning Ca\(^{2+}\) influx via the NCX. On the basis of thermodynamic considerations, Ca\(^{2+}\) influx via NCX is most likely to occur during the very early phase of the action potential (AP). Normally, little Ca\(^{2+}\) enters via the NCX, but Ca\(^{2+}\) entry can increase greatly when intracellular [Na\(^{+}\)] ([Na\(^{+}\)]) increases; this can occur as a result of Na\(^{+}/K^{+}\) ATPase inhibition during ischemia and reperfusion.

Ca\(^{2+}\) entry via NCX during the AP, although normally small, could be sufficient to trigger Ca\(^{2+}\) release from the sarcoplasmic reticulum (SR), because depolarization increases the driving force for Ca\(^{2+}\) entry via NCX. Ca\(^{2+}\) entry can also occur when the Na\(^{+}\) current causes local subsarcolemmal [Na\(^{+}\)], to rise. However, the physiological relevance of SR Ca\(^{2+}\) release triggered by NCX is controversial.

Inhibition of Na\(^{+}/K^{+}\) ATPase by cardiac glycosides causes [Na\(^{+}\)], to increase, resulting in increased cell Ca\(^{2+}\) load via NCX. Positive inotropy or even Ca\(^{2+}\) overload and arrhythmias can result. Strophanthidin increases intracellular [Ca\(^{2+}\)] ([Ca\(^{2+}\)]) and Ca\(^{2+}\) transients as [Na\(^{+}\)] rises; when arrhythmias occur, Ca\(^{2+}\) transient amplitude decreases while [Na\(^{+}\)], and basal [Ca\(^{2+}\)], continue to increase. An increase of [Ca\(^{2+}\)], in response to increased [Na\(^{+}\)], can occur because either Ca\(^{2+}\) efflux via the NCX is reduced (failing to match Ca\(^{2+}\) influx) or [Na\(^{+}\)], levels are high enough (NCX mediates net Ca\(^{2+}\) influx).

KB-R7943 (KBR) is a novel agent that reportedly preferentially blocks the Ca\(^{2+}\) influx mode of the cardiac NCX rather than the Ca\(^{2+}\) extrusion mode. We used this property of KBR to examine the likely role of Ca\(^{2+}\) influx via NCX in cellular Ca\(^{2+}\) handling during excitation-contraction (E-C) coupling and during the genesis of strophanthidin-induced inotropy and Ca\(^{2+}\) overload. KBR had no effect on
normal E-C coupling, but it blocked Ca\textsuperscript{2+} entry via NCX. KBR also blocked the spontaneous activity caused by strophanthidin-induced increased [Na\textsuperscript{+}], without preventing the inotropy. Thus, inotropy can be due to a reduction of Ca\textsuperscript{2+} efflux via NCX, whereas Ca\textsuperscript{2+} overload may require net Ca\textsuperscript{2+} influx.

**Methods**

**Preparation of Ventricular Myocytes**

Ventricular myocytes were isolated from male Sprague-Dawley rats (weighing 200 to 240 g) and loaded with indo-1-acetoxymethylester at 23°C.\textsuperscript{14} Then, they were placed in a chamber on the stage of an inverted microscope (TMD, Nikon) with a perfusate containing (in mmol/L): NaCl 137, KCl 4, MgSO\textsubscript{4} 1.2, glucose 10, HEPES 10, and CaCl\textsubscript{2} 1.5 (pH adjusted to 7.4 with NaOH). Myocytes were field-stimulated through Ag-AgCl electrodes at 0.5 Hz.

**Apparatus**

The indo-1 fluorescence ratio (405/485 nm emission with 340 nm excitation)\textsuperscript{2} was used after background subtraction to measure [Ca\textsuperscript{2+}], using the following formula:

\[
\text{[Ca}^{2+}] \text{= } K_i \times (S_{SB} \times (R - R_{min})/(R_{max} - R)
\]

R\textsubscript{max} and R\textsubscript{min} (maximum and minimum indo-1 fluorescence ratios) were determined in cells exposed to ionomycin (10 μmol/L) and either CaCl\textsubscript{2} (5 mmol/L) or EGTA (10 mmol/L), respectively. K\textsubscript{i} equaled 844 mmol/L,\textsuperscript{19} and S\textsubscript{SB}, the ratio of Ca-free to Ca-bound 485-nm fluorescence, was 1.096. Contraction was measured using an edge detection system (Hamamatsu Photonics) with transilluminated red light (>600 nm).

**AP Recording and Analysis**

An Axopatch 200A amplifier (Axon Instruments) was used in current-clamp mode to record APs in a perforated patch configuration (extracellular Ca\textsuperscript{2+} concentration was 1 mmol/L). Pipettes (1 to 1.5 meqohms) were filled with the following (in mmol/L): KCl 40, K-glutamate 80, NaCl 0 or 10, and HEPES 10 (pH 7.2); amphotericin B (120 μg/mL) was added only in the backfill. APs were evoked by injecting a depolarizing current (0.5 ms, 1.25 threshold) via the recording electrodes (access resistance, 4 to 7 meqohms). AP durations at 50% and 90% repolarization (APD\textsubscript{50} and APD\textsubscript{90}) were measured from synchronized averages of 10 to 20 steady-state APs.

**Test Reagent**

The KBR was a gift from the New Drug Research Laboratories, Kanebo Co, Ltd (Osaka, Japan), and stock solution was dissolved in dimethyl sulfoxide in 5 or 10 μmol/L KBR was ≤0.1%.

**Statistical Analyses**

Results were expressed as mean±SEM for the number of isolated myocytes. Student’s t test or 1-way ANOVA were used for analyses; P<0.05 was considered significant.

**Results**

**KBR Blocks Ca\textsuperscript{2+} Influx but Not Ca\textsuperscript{2+} Efflux**

For the control experiments (shown in Figure 1) only, SR function was completely blocked by pretreatment with 10 μmol/L ryanodine and 1 μmol/L thapsigargin.

To assess Ca\textsuperscript{2+} influx via NCX, extracellular Na\textsuperscript{+} was abruptly removed (replaced by tetramethylammonium) for 15 s, which changed the thermodynamic driving force on NCX to favor Ca\textsuperscript{2+} influx. Figure 1A shows that this caused [Ca\textsuperscript{2+}] to rise continuously, as was expected for Ca\textsuperscript{2+} entry via NCX. When extracellular Na\textsuperscript{+} was returned, [Ca\textsuperscript{2+}] recovered with a similar time course. KBR dose-dependently inhibited the Na-free–induced increase in [Ca\textsuperscript{2+}], (measured at 15 s). Washout of KBR effects was slow (>30 minutes) and often incomplete.

To test KBR effects on Ca\textsuperscript{2+} influx via NCX, twitch were activated so that [Ca\textsuperscript{2+}] decline was almost entirely due to NCX efflux. Figure 1C shows that with 5 μmol/L KBR, Ca\textsuperscript{2+} influx was quantified by % inhibition of 0Na-induced rise in [Ca\textsuperscript{2+}]. Figure 1B, indicated with a similar control data were obtained at 36°C, where 5 μmol/L KBR could be used to selectively inhibit Ca\textsuperscript{2+} influx via NCX, whereas Ca\textsuperscript{2+} efflux via NCX was unaffected (111±13% of control rate constant of [Ca\textsuperscript{2+}], decline). Simultaneous control data were obtained at 36°C, where 5 μmol/L KBR depressed peak [Ca\textsuperscript{2+}], on Na\textsuperscript{+} removal (to 19.9±6.4% of control, n=3) but left the NCX-mediated rate of [Ca\textsuperscript{2+}], decline unchanged (94±16% of control, n=4). We concluded that 5 μmol/L KBR could be used to selectively inhibit Ca\textsuperscript{2+} influx via NCX in subsequent experiments.

**KBR Does Not Affect Twitch Contractions or Ca\textsuperscript{2+} Transients at 23°C**

To test whether Ca\textsuperscript{2+} influx via NCX is physiologically important, we measured the effects of KBR on 0.5-Hz twitch
contractions, both steady-state (SS) and after 30 s of rest (PR), and Ca\(^{2+}\) transients. Figure 2 shows that 5 \(\mu\)mol/L KBR (given over 8 minutes) did not alter twitch Ca\(^{2+}\) transients or contractions (whether SS or PR, \(n=12\); see the Table).

Figure 3A shows that although peak twitch [Ca\(^{2+}\)], was not altered by KBR, [Ca\(^{2+}\)], decline was slightly slowed (\(P<0.01\), paired \(t\) test, \(n=12\) cells; see the Table). This slowing of twitch [Ca\(^{2+}\)], decay with KBR was not seen when cells were depleted of [Na\(^+\)], and studied with Li\(^+\) replacing extracellular Na\(^+\) (thereby blocking NCX; data not shown). This indicates that NCX is responsible for the slower twitch [Ca\(^{2+}\)], decline with KBR. Without external Na\(^+\), [Ca\(^{2+}\)], decline is almost entirely due to the SR Ca-ATPase, which suggests that SR Ca\(^{2+}\) transport is not altered by KBR.

Caffeine-induced contractures and Ca\(^{2+}\) transients were also used to assess SR Ca\(^{2+}\) content (Figure 3B). Caffeine (10 mmol/L) was rapidly applied in Na\(^+\)-free (tetramethylammonium-substituted), Ca\(^{2+}\)-free, 1 mmol/L EGTA solution (to block NCX). KBR (5 \(\mu\)mol/L) did not affect the amplitude (Table) or other properties (data not shown) of caffeine-induced Ca\(^{2+}\) transients (\(n=8\) cells).

**KBR Prolongs the Low Plateau of the AP**

Figures 4A and 4B show that APs, recorded in SS at 0.5 Hz, were reversibly prolonged by KBR in the late low plateau phase (when Ca\(^{2+}\) influx via NCX is not expected). Moreover, the same prolongation of the low plateau was still seen whether pipette [Na\(^+\)] was 0 or 10 mmol/L (Figure 4, A versus B). This suggests that the AP prolongation was not due to altered NCX current. The APD\(_{50}\) was not significantly changed by KBR (\(n=8\)), whereas the APD\(_{50}\) was increased by KBR from 74.5 \pm 10.0 ms to 123.8 \pm 17.5 ms (\(P<0.05\)). These effects began in several seconds, maximized within 3 to 4 minutes, and were reversible with a similar time course. The resting potential (–79.6 \pm 2.6 mV) depolarized by 0.96 mV with KBR in 8 cells (not significant).

**KBR Does Not Affect Twitch Contractions or Ca\(^{2+}\) Transients at 36°C**

NCX current is temperature-dependent, and the role of Ca\(^{2+}\) entry via NCX in triggering SR Ca\(^{2+}\) release may be greater at 37°C than at 23°C to 25°C. Therefore, we repeated the above experiments at 36°C, but KBR still failed to change the amplitudes of Ca\(^{2+}\) transients or twitches, either SS or PR (Table).

These data suggest that under physiological conditions in rat ventricular myocytes, Ca\(^{2+}\) influx via NCX does not modulate SS contractions. Our data further argue against the hypothesis that the PR potentiation observed in the rat (and some other species) is due to Ca\(^{2+}\) influx via NCX. The data do not address Ca\(^{2+}\) efflux via the NCX, because 5 \(\mu\)mol/L KBR did not affect NCX-mediated Ca\(^{2+}\) extrusion.

**KBR Reduces Spontaneous Activity During Na\(^+\) Loading**

Figure 5 shows the effect of 50 \(\mu\)mol/L strophanthidin on cell contraction and [Ca\(^{2+}\)], during SS 0.5 Hz stimulation. Blocking Na\(^+/K\(^+\) ATPase with strophanthidin should cause [Na\(^+\)] to rise gradually, shifting the thermodynamic balance on NCX toward Ca\(^{2+}\) entry. In all 16 cells studied, strophanthidin increased both twitch contraction and Ca\(^{2+}\) transient amplitudes. In 4 of these cells (Figure 5), spontaneous contractions and [Ca\(^{2+}\)], oscillations were suppressed (Figure 5, C), indicating that Ca\(^{2+}\) release via NCX was reduced by KBR. The amplitude and duration of Ca\(^{2+}\) transients also decreased, consistent with reduced NCX current. These data suggest a mechanism to explain the negative inotropic effect of KBR observed in the rat heart in vivo.
tions occurred within ~10 minutes, indicating Ca\(^{2+}\) overload; also, in these cells, twitch contractions decreased (Figure 5Ac).

In all 4 of these cells, 5 \(\mu\)mol/L KBR abolished the spontaneous activity (Figure 5Bd) but left the strophanthidin-induced inotropy largely intact (compare Figure 5B, b and d). KBR also partially restored diastolic cell length.

In the 12 cells that did not show spontaneity, KBR reduced Ca\(^{2+}\) transients toward predrug control amplitude in only 3 cells; the same inotropic state was sustained in the other 9 cells. Furthermore, as shown in Figure 6, the application of 5 \(\mu\)mol/L KBR 5 minutes before strophanthidin administration did not prevent the increase in amplitude of twitch contractions and Ca\(^{2+}\) overload was not observed.

The failure of KBR to block strophanthidin inotropy suggests that Ca\(^{2+}\) influx via NCX is not essential for the positive inotropic effect of strophanthidin. That is, simply reducing Ca\(^{2+}\) efflux (due to increased [Na\(^{+}\)]) is sufficient. However, insofar as spontaneous activity is a marker of a transition to Ca\(^{2+}\) overload in response to elevated [Na\(^{+}\)], the block of all spontaneous activity by KBR suggests that Ca\(^{2+}\) influx via NCX may cause the overload.

Discussion

KBR as a Tool to Study NCX

KBR can selectively inhibit Ca\(^{2+}\) influx (versus efflux) via NCX under appropriate conditions. In guinea pig ventricular myocytes, KBR inhibited 50% of outward and inward NCX current at 0.3 and 17 \(\mu\)mol/L, respectively.\(^{16}\) Directional selectivity was less in sarcosomal vesicles,\(^{17}\) and there may be extracellular Ca\(^{2+}\) sensitivity to the block.\(^{17,21,22}\) Although the mechanism of directional selectivity is not clear, Figure 1 shows the selective block of Ca\(^{2+}\) influx via NCX by 5 \(\mu\)mol/L KBR under our conditions.

Ca\(^{2+}\) Influx Via NCX and E-C Coupling

The lack of effect of KBR on normal E-C coupling (Table and Figures 2 through 4) is not consistent with a role for Ca\(^{2+}\)

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**Figure 3.** SS twitch and caffeine-induced Ca\(^{2+}\) transient amplitudes are unaltered by KBR. A, SS twitch Ca\(^{2+}\) transients; B, caffeine-induced Ca\(^{2+}\) transients. \(\times\)KBR at 23°C. TTP indicates time to peak [Ca\(^{2+}\)]; \(\tau\), time constant of [Ca\(^{2+}\)] decline. In B, perfusate [Ca\(^{2+}\)] was reduced to 0.8 mmol/L to prevent Ca\(^{2+}\) overload.

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**Figure 4.** KBR lengthens only low plateau of rat ventricular AP. APs were evoked by injecting inward current at 0.5 Hz and recorded in whole cell perforated patch at 23°C. A and B, APs with 10 mmol/L and 0 mmol/L pipette [Na\(^{+}\)], respectively (latter blocks NCX-mediated Ca\(^{2+}\) influx). 1.5 \(\mu\)mol/L KBR. C, Mean APD\(_{50}\) and APD\(_{90}\). Results are mean\(\pm\)SEM from 8 experiments. *P<0.05 by paired t test.

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**Figure 5.** KBR blocks strophanthidin-induced arrhythmia but not inotropy. A, Continuous recording of twitch cell shortening from typical experiment with 50 \(\mu\)mol/L strophanthidin and 5 \(\mu\)mol/L KBR added as indicated by bars. B, Ca\(^{2+}\) transients recorded during control perfusion (a), 5 minutes after starting strophanthidin perfusion (b), an then in arrhythmia (c) and 2 minutes after addition of KBR (d).
entry via NCX. Compared with the L-type Ca\(^{2+}\) channel, NCX mediates Ca\(^{2+}\) entry, which is smaller and slower, making it a less efficient trigger for SR Ca\(^{2+}\) release, even for a given Ca\(^{2+}\) influx.\(^{14}\) This may be because (1) NCX does not couple tightly with the SR Ca\(^{2+}\) release channel, as does the L-type Ca\(^{2+}\) channel, and (2) 1000 NCX molecules are required to produce the flux of one L-type Ca\(^{2+}\) channel. We conclude that the role of Ca\(^{2+}\) entry via NCX is minimal in rat ventricular E-C coupling.

Ca\(^{2+}\) entry via NCX can occur during the cardiac AP upstroke, when the membrane potential (E\(_{m}\)) transiently exceeds the NCX reversal potential.\(^{23}\) Indeed Ca\(^{2+}\) can enter via NCX during depolarizations and can trigger SR Ca\(^{2+}\) release,\(^{1,4,6,7,9,10}\) although evidence is most compelling when the Ca\(^{2+}\) current is blocked. If an L-type Ca\(^{2+}\) channel opens, local [Ca\(^{2+}\)] around the NCX will rapidly become high enough to prevent net Ca\(^{2+}\) entry by NCX. This occurs very early in the AP because of the rapid activation of Ca\(^{2+}\) channels. Tetrodotoxin-sensitive Na\(^{+}\) influx could raise sarcolemmal [Na\(^{+}\)], sufficiently to promote Ca\(^{2+}\) entry via NCX and trigger Ca\(^{2+}\) release,\(^{11,12}\) but this remains controversial.\(^{13,24,25}\)

Lower temperature reportedly limits the activation of SR Ca\(^{2+}\) release by NCX\(^{9,26}\); however, we found no E-C coupling depression at 23°C or 36°C. Species differences could alter the effectiveness of Ca\(^{2+}\) entry via NCX. This remains possible: we found KBR (5 \(\mu\)mol/L) slightly reduced contractions and Ca\(^{2+}\) transients in guinea pig myocytes (unpublished data). In transgenic mice overexpressing NCX current \(\approx 3\)-fold, evidence supporting NCX-induced SR Ca\(^{2+}\) release is conflicting.\(^{27,28}\)

A limitation in previous work was a difficulty in inhibiting Ca\(^{2+}\) entry via NCX without altering Ca\(^{2+}\) current. KBR may be an imperfect agent, but our results in rat ventricular myocytes clearly indicate that no E-C coupling change occurred when blocking Ca\(^{2+}\) entry via NCX.

Potentiation of PR contractions and Ca\(^{2+}\) transients in the rat\(^{29}\) could result from Ca\(^{2+}\) entry via NCX loading SR, secondary to [Na\(^{+}\)], elevation, after a train of stimuli.\(^{1,23}\) Resuming stimulation could then give a negative staircase as cell and SR Ca\(^{2+}\) content decline.\(^{1,3,23}\) Because PR potentiation was unaltered by KBR (Figure 2A and the Table), Ca\(^{2+}\) influx via NCX does not explain PR potentiation. PR potentiation also occurs without increased SR Ca\(^{2+}\) load or Ca\(^{2+}\) current.\(^{29,30}\) This is likely due to a slow recovery of SR Ca\(^{2+}\) release channels after an activated release.\(^{29,31,32}\)

**Changes in AP**

KBR lengthened the low AP plateau (Figure 4), reflecting either less outward current and/or greater inward current. Although the inhibition of outward NCX current by KBR is in the correct direction, it is unlikely that NCX current is outward during the low plateau of the AP (or at rest).\(^{1}\) Moreover, because these same KBR effects were seen with Na\(^{+}\)-free pipette solution, NCX is probably not involved.

Although KBR blocks outward NCX current, it blocks other currents at higher concentrations.\(^{36}\) Preliminary perforated patch voltage clamp recordings made during our AP recordings suggest some decrease in composite outward K\(^{+}\) and inward Ca\(^{2+}\) currents (data not shown). More work is needed to clarify which currents cause the AP changes, but modest inhibition of K\(^{+}\) current could explain both a depolarized resting E\(_{m}\) and the longer AP.

Prolongation of the late phase of the AP with KBR is unlikely to alter SR Ca\(^{2+}\) release because (1) Ca\(^{2+}\) release is independent of duration after \(\approx 20\) ms\(^{1,33,34}\) and (2) the AP differences are at an E\(_{m}\) that does not activate SR Ca\(^{2+}\) release. However, the slower repolarization with KBR could contribute to the slower twitch [Ca\(^{2+}\)], decline, because Ca\(^{2+}\) extrusion by NCX is slower at a more positive E\(_{m}\) (and the NCX reversal potential is rather negative in the rat).\(^{1}\) This could slow Ca\(^{2+}\) extrusion, even if the total amount extruded was unchanged (such that SR Ca\(^{2+}\) content is unchanged, as observed).

In some cells, KBR reduced the peak of the AP (Figure 4A). This could be explained by a small reduction in Na\(^{+}\) current,\(^{36}\) which could also explain the higher AP threshold with KBR (data not shown).

**Ca\(^{2+}\) Influx Via NCX Under Na\(^{+}\) loading:**

**Inotropy and Ca\(^{2+}\) Overload**

Na\(^{-}/K\) pump blockade by cardiac glycosides increases [Na\(^{+}\)], which enhances contractility by increasing cellular Ca\(^{2+}\) due to NCX.\(^{1,15}\) This could simply be caused by reduced Ca\(^{2+}\) efflux by the NCX as [Na\(^{+}\)] rises. That is, less Ca\(^{2+}\) efflux for a given Ca\(^{2+}\) influx would increase cell Ca\(^{2+}\). The increased [Na\(^{+}\)], could also increase Ca\(^{2+}\) influx via NCX. The apparent preferential block of Ca\(^{2+}\) influx via NCX by KBR allows unique insights into this functional distinction.

Our results indicate that Ca\(^{2+}\) influx via NCX is not important under normal conditions in the rat ventricle, although resting [Na\(^{+}\)], in the rat is high compared with the rabbit or guinea pig ventricle.\(^{23,35}\) However, as [Na\(^{+}\)] increases, Ca\(^{2+}\) influx is more favored and Ca\(^{2+}\) efflux is less favored thermodynamically. The ability of KBR to block oscillations attributable to Ca\(^{2+}\) overload, without preventing the inotropic effect of strophanthidin, leads us to propose that inhibiting Ca\(^{2+}\) influx via NCX is sufficient to produce the inotropic effect of cardiac glycosides. In contrast, the arrhythmogenic effects of glycosides may depend on [Na\(^{+}\)], rising high enough to cause the net Ca\(^{2+}\) entry via NCX to be favored.
For a resting [Na⁺], level of 12 to 14 mmol/L and a [Ca²⁺], level of 100 mmol/L, the predicted NCX reversal potential is −67 to −79 mV; thus, Ca²⁺ influx at rest is slightly favored. If [Na⁺], rose by just 3 mmol/L, the reversal potential would become −85 to −95 mV, negative to resting Eₐ and favoring net Ca²⁺ influx at rest and Ca²⁺ overload. Spontaneous Ca²⁺ release at the resting Eₐ would drive Ca²⁺ influx (lessening Ca²⁺ overload), but also produce transient inward NCX current delayed afterdepolarization, and triggered arrhythmias.¹

Santana et al.³⁷ recently suggested that glycosides cause Ca²⁺ influx through Na⁺ channels. However, this possibility remains controversial,³⁸ and we found that glycoside inotropy does not occur without a functioning NCX.³⁹ Thus, this possibility is unlikely to complicate our interpretations.

We conclude that in rat ventricular myocytes, Ca²⁺ influx via NCX is not important in SS switches, rest potentiation, or glycoside inotropy. However, KBR can prevent the arrhythmogenic effects associated with glycoside toxicity (which may rely on net Ca²⁺ influx via NCX). This latter effect of KBR makes it a potentially useful adjunct to digitalis treatment and justifies further investigation.

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