The Amiodarone Derivative KB130015 [2-Methyl-3-(3,5-diiodo-4-carboxymethoxybenzyl)benzofuran] Induces an Na⁺-Dependent Increase of \([\text{Ca}^{2+}]\) in Ventricular Myocytes

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ABSTRACT

KB130015 \([\text{KB}: 2\text{-methyl}-3\text{-}(3,5\text{-diiodo}-4\text{-carboxymethoxybenzyl})\text{benzofuran}]\) is a novel amiodarone derivative designed to retain the antiarrhythmic effects without the side effects. Unlike amiodarone, KB slows Na⁺ current inactivation and could, via an increase in [Na⁺], potentially lead to \(\text{Ca}^{2+}\) overload. Therefore, we studied the effects of KB on Na⁺ and \(\text{Ca}^{2+}\) handling in single pig ventricular myocytes using the whole-cell ruptured patch-clamp technique and \(\text{K}_\text{f}\)-fluo-3 as \([\text{Ca}^{2+}]\) indicator. KB at 10 \(\mu\)M did not prolong action potential duration but slightly increased the early plateau; spontaneous afterdepolarizations were not observed. The amplitude of the \([\text{Ca}^{2+}]\) transient was larger (434.9 ± 57.2 versus 326.8 ± 39.8 nM at baseline, \(n = 13\), \(P < 0.05\)), and the time to peak \([\text{Ca}^{2+}]\) was prolonged. During voltage-clamp pulses, \([\text{Ca}^{2+}]\), transient peak was also larger (578.1 ± 98.9 versus 346.4 ± 52.6 nM at baseline, \(P < 0.05\)). Although L-type \(\text{Ca}^{2+}\) current was reduced (by 21.9% at \(+10\) mV, \(n = 9\), \(P < 0.05\)), sarcoplasmatic reticulum \(\text{Ca}^{2+}\) content was significantly enhanced with KB. Forward \(\text{Na}^+\)/\(\text{Ca}^{2+}\) exchange was significantly decreased after KB application, but reverse mode of the \(\text{Na}^+\)/\(\text{Ca}^{2+}\) exchanger was significantly larger, suggesting an increase in \([\text{Na}^+]\), with KB. This was confirmed by a 2-fold increase of the \([\text{Na}^+]\)-dependent current generated by the Na/K-ATPase (from 0.17 ± 0.02 to 0.38 ± 0.06 pA/pF, \(P < 0.05\)). In conclusion, as predicted from the slowing of \(I_{\text{Na}}\) inactivation, KB130015 leads to an increase in \([\text{Na}^+]\), and consequently in cellular \(\text{Ca}^{2+}\) load. This effect is partially offset by a decrease in \(I_{\text{Ca}}\) resulting in a mild inotropic effect without the signs of \(\text{Ca}^{2+}\) overload and related arrhythmias usually associated with \(\text{Na}^+\) channel openers.

Sudden cardiac death, mostly related to arrhythmias, is a major cause of mortality (Zipes and Wellens, 1998; Richter et al., 2005). Lethal arrhythmias occur in many cardiac diseases, including congenital ion channel mutations without structural heart disease as well as genetic and acquired cardiomyopathies. Particularly in the setting of ischemic cardiomyopathy and heart failure, mortality due to ventricular arrhythmias is high, and the search for efficient antiarrhythmic drugs has been frustrating. The recent introduction of device therapy with an implantable cardiac defibrillator (ICD) has been a major success in improving survival. It was superior to any medical pharmacotherapy with the ICD can substantially reduce the number of electroshocks, thus improving efficiency of treatment and quality of life.

The major drawback of current antiarrhythmics is the earlier experiences of increased mortality in the CAST studies (CAST Investigators, 1989). The newer class III agents with a pure K⁺ channel blocking action have also been associated with proarrhythmia, notably a high incidence of Torsade-de-Pointes in the SWORD study (Waldo et al., 1996). They are not a first choice for ventricular arrhythmias occurring in the setting of ischemic cardiomyopathy where there is often already prolongation of the action potential at baseline. The multiaction drug amiodarone has not been reported to have the proarrhythmic effect of other class III agents (Amiodarone Trials Meta-Analysis Investigators, 1997). It has specific effects on multiple cardiac channel openers.
diac ion channels that differ in acute and chronic treatment (Kodama et al., 1997). After myocardial infarction, treatment with amiodarone has been reported to prevent arrhythmic death (Cairns et al., 1997; Julian et al., 1997), but a recent trial in heart failure patients confirmed superiority of ICD (Bardy et al., 2005). Because of its low proarrhythmic potential, amiodarone remains a widely used and efficient drug. However, its long-term clinical use is limited by its significant extracardiac toxicity. Because of close structural similarity with thyroid hormones, symptoms of hypothyroidism can occur. Corneal deposits and development of lung fibrosis can be major reasons for discontinuation of amiodarone treatment (Martin, 1990).

Therefore, several compounds have been developed based on the amiodarone structure, aiming to retain the efficiency and cardiac safety of amiodarone, with less of the extra-cardiac side effects. KB130015 (KB) is one such new drug (Carlsson et al., 2002). Preliminary data in guinea pig suggest that KB has a toxicity profile more advantageous that amiodarone. In the same study in guinea pig papillary muscle, KB has been shown to prolong action potential duration suggesting a potential antiarrhythmic effect. Like amiodarone, it acts on many ion channels, including Ca$$^{2+}$$, Na$$^+$$, and K$$^-$$ channels (for review, see Mubagwa et al., 2003). Unlike amiodarone, KB slows the inactivation of voltage-dependent Na$$^+$$ channels (Macianskiene et al., 2003b). This effect is expected to increase intracellular [Na$$^+$$] and thus the cellular Ca$$^{2+}$$ load through an increased influx via Na$$^+$/Ca$$^{2+}$$ exchange, a potentially positive inotropic effect. However, KB also decreases L-type Ca$$^{2+}$$ current, which would lower the cellular Ca$$^{2+}$$ load (Macianskiene et al., 2003a).

The net effect of these opposite actions is yet unknown and is of considerable interest given the earlier experience of increased mortality during chronic treatment in patients with heart failure with agents that increased cellular Ca$$^{2+}$$ (Packer, 1993). Preliminary data indicated that KB increased cell shortening, especially at high concentrations (Mubagwa et al., 2003). Therefore, in the present study, we examined the effect of KB on cellular Ca$$^{2+}$$ load and Na$$^+$/Ca$$^{2+}$$ exchange function associated with alterations in increased Na$$^+$$ influx. We used pig ventricular myocytes that have action potential duration and frequency behavior close to that of humans.

Materials and Methods

Cell Isolation. Single left ventricular myocytes were enzymatically isolated from the hearts of domestic pigs of either sex (body weight, 41 ± 4 kg; n = 10) as previously described (Stankovicová et al., 2000). We used only cells isolated from the midmyocardial layer, mostly from the left ventricular anterior free wall, but in a number of experiments also from the left ventricular posterior wall. Cells were stored at room temperature and used within 24 h.

Solutions and Drugs. All experiments were performed in normal Tyrode’s solution 137 mM NaCl, 5.4 mM KCl, 0.5 mM MgCl$$_2$$, 1.8 mM CaCl$$_2$$, 11.8 mM Na-Hepes, and 10 mM glucose 10, pH 7.40. The pipette solution for whole-cell patch clamp contained 120 mM K-aspartate, 10 mM NaCl, 20 mM KCl, 10 mM K-Hepes, 5 mM MgATP, and 0.05 mM K$$_2$$HPO$$_4$$, pH 7.2.

A stock solution of KB130015 (a gift from Karo-Bio, Huddinge, Sweden) was prepared in dimethyl sulfoxide at a concentration of 10 mM. The final concentration to be used was obtained by diluting 1:1000 (v/v) in normal Tyrode’s solution. A 1:1000 dilution of the solvent in normal Tyrode’s solution had no effects by itself ($n_{cell} = 9$, data not shown). NiCl$$_2$$ (Sigma-Aldrich, St. Louis, MO) was dissolved directly in the Tyrode’s solution at 2.5 mM. Dihydrouabain (DHO; Sigma-Aldrich) was prepared as a 1 mM stock in distilled water and diluted 1:100 in the Tyrode’s solution before use.

Experimental Setup. [Ca$$^{2+}$$], transients were measured with fluo-3 as a [Ca$$^{2+}$$] indicator. The setup for fluorescence and membrane currents recording was as described before (Antoons et al., 2002). The fluorescence signals were corrected for the background recorded after the seal formation. This signal was further calibrated to [Ca$$^{2+}$$], values, using an F$$\text{F}$$ reading obtained at the end of the experiment (Trafford et al., 1999; Antoons et al., 2002).

Experimental Protocols. We recorded action potentials and [Ca$$^{2+}$$]$$^\text{i}$$ transients in current clamp mode, applying 5-ms current injections to trigger action potentials. During voltage-clamp experiments, the holding potential was −70 mV. For measuring L-type Ca$$^{2+}$$ current, I$$_{\text{Ca,L}}$$, the Na$$^+$$ current was inactivated by a prepulse to −50 mV. I$$_{\text{Ca,L}}$$ was taken as the difference between the peak inward current and the current at the end of the pulse during depolarizations to −40 up to +50 mV. The Ca$$^{2+}$$ content of the sarcoplasmic reticulum (SR) was measured during a fast application of 10 mM caffeine at a holding potential of −50 mV following a train of 10 conditioning pulses from −70 to +10 mV at 1 Hz.

The amplitude of the reverse mode of Na$$^+$/Ca$$^{2+}$$ exchange current was measured as the nickel-sensitive current during a depolarizing step to +40 mV. The current generated by the Na$$^+$/ATPase was measured as DHO-sensitive current at a holding potential of −50 mV following a train of 10 conditioning pulses from −70 to +10 mV at 1 Hz. All the experiments were done at 37°C.

Statistics. All data are shown as mean ± S.E.M. As statistical test, a paired Student’s t test was used for single measurements, and ANOVA was used for multiple measurements.

Results

KB Effects on the [Ca$$^{2+}$$]$$^\text{i}$$ Transient during Action Potentials. Figure 1A illustrates the effect of KB on the action potential and the pooled data from 13 myocytes. On average, KB did not prolong the action potential duration at 90% of repolarization [318.8 ± 25.9 versus 352.5 ± 23 ms in control (CTRL)]. Resting membrane potential was unchanged, but the early repolarization tended to be less with an increase of V$$\text{m}$$ at 15 ms in 10 of 13 cells. Figure 1B illustrates the corresponding effect on the [Ca$$^{2+}$$]$$^\text{i}$$ transient with mean data for [Ca$$^{2+}$$]$$^\text{i}$$ at rest and at peak. Resting [Ca$$^{2+}$$]$$^\text{i}$$ was significantly enhanced with KB (182.2 ± 18.9 versus 121.8 ± 12.7 nM in CTRL, $n_{cell} = 25$, P < 0.01), and the amplitude of the [Ca$$^{2+}$$]$$^\text{i}$$ transient was increased (peak [Ca$$^{2+}$$]$$^\text{i}$$ = 434.9 ± 37.2 versus 326.8 ± 39.8 nM in CTRL, P < 0.05). The kinetics of the [Ca$$^{2+}$$]$$^\text{i}$$ transient are given in Fig. 1C. Time to peak was significantly prolonged, and the time from onset of depolarization to half-relaxation was also prolonged, although the rate of relaxation itself was not altered.

KB Effects on the [Ca$$^{2+}$$]$$^\text{i}$$ Transient during Depolarizing Steps of Fixed Duration and Amplitude. To exclude the secondary effects of changes in action potential profile on Ca$$^{2+}$$ handling, we examined the [Ca$$^{2+}$$]$$^\text{i}$$ transients during a single voltage step of 250 ms from −70 to +10 mV, illustrated in Fig. 2. As during action potentials, peak [Ca$$^{2+}$$]$$^\text{i}$$, was significantly larger with KB (578.1 ± 98.9 versus 346.4 ± 52.6 nM in CTRL, P < 0.05), and the rise of the [Ca$$^{2+}$$]$$^\text{i}$$ transient was slower (time to peak 195.9 ± 11.8 versus 130.3 ± 9.2 ms in CTRL, P < 0.01). Rate of relaxation was unchanged.

KB Reduces I$$_{\text{Ca,L}}$$, but Increases Ca$$^{2+}$$ Release. To examine the role of I$$_{\text{Ca,L}}$$ in the increased [Ca$$^{2+}$$]$$^\text{i}$$ transient, we subsequently used a double-step protocol to separate the Na$$^+$$ current, I$$\text{Na}$$, and the L-type Ca$$^{2+}$$ current, I$$_{\text{Ca,L}}$$. This approach is illustrated in Fig. 3A. A step from −70 to −50 mV activated I$$\text{Na}$$ and was followed by a step to +10 mV, activat-
ing $I_{\text{CaL}}$. As expected, KB slowed $I_{\text{Na}}$ inactivation as manifested in the current trace during the step to $-50$ mV. This step, which in control induced a small $[\text{Ca}^{2+}]_i$ transient most likely related to spurious activation of $\text{Ca}^{2+}$ channels (Sipido et al., 1995), now had a large $[\text{Ca}^{2+}]_i$ transient in the presence of KB. During the step to $+10$ mV, we saw a decrease of $I_{\text{CaL}}$, but the amplitude of the accompanying $[\text{Ca}^{2+}]_i$ transient was significantly increased. Figure 3B summarizes the data on the $[\text{Ca}^{2+}]_i$ transients during the step to $+10$ mV and shows that time to peak was unchanged.

In a similar protocol as in Fig. 3A, we studied further the effect of KB on the amplitude and the voltage dependence of $I_{\text{CaL}}$ by varying the amplitude of the second depolarizing step. KB decreased significantly the amplitude of $I_{\text{CaL}}$ (by $21\%$ at $+10$ mV, $n_{\text{cells}} = 9$, $P < 0.05$) without affecting its voltage dependence (Fig. 4A). Despite the reduction in $I_{\text{CaL}}$, KB significantly increased the amplitude of the associated $[\text{Ca}^{2+}]_i$ transient (at $+10$ mV, peak $[\text{Ca}^{2+}]_i$ transient $419.9 \pm 46.4$ versus $302.6 \pm 58.6$ nM in CTRL, $P < 0.05$) (Fig. 4B). Note that this effect is smaller than what is seen during a single step from $-70$ to $+10$ mV (Fig. 3B).

A decrease in $I_{\text{CaL}}$ associated with an enhanced $\text{Ca}^{2+}$ release suggests an increase in $\text{Ca}^{2+}$ availability in the SR. Therefore, we estimated SR $\text{Ca}^{2+}$ content by emptying the SR with a fast application of 10 mM caffeine (Fig. 5A). The amplitude of the $[\text{Ca}^{2+}]_i$ transient, measured as the difference between the peak $[\text{Ca}^{2+}]_i$ and diastolic $[\text{Ca}^{2+}]_i$, was significantly larger with KB (670.9 $\pm$ 163.6 versus 345.1 $\pm$ 56.7 nM in CTRL, $P < 0.05$) consistent with a larger SR $\text{Ca}^{2+}$ content (Fig. 5B). However, the integrated $\text{Na}^+$/Ca$^{2+}$ exchange current, which is normally a precise indicator for the amount of $\text{Ca}^{2+}$ released from the SR (Varro...
et al., 1993) was not significantly increased (Fig. 5C). This can only be explained by an altered Na⁺/Ca²⁺ exchanger function and decreased removal of Ca²⁺ by the (forward mode) of the Na⁺/Ca²⁺ exchanger. Consistent with this hypothesis, the rate of relaxation of the caffeine-induced [Ca²⁺], transient was much slower in the presence of KB (time constant of [Ca²⁺], decay 1624.5 ± 133.1 versus 1186.8 ± 60.2 ms in CTRL, n_cells = 10, P < 0.01) (Fig. 5D). Likewise, peak Na⁺/Ca²⁺ exchange current was reduced (−1.34 ± 0.13 versus −1.71 ± 0.12 pA/pF in CTRL, P < 0.05) (Fig. 5E) and declined with a longer time constant (448.6 ± 38.3 versus 346.4 ± 31.1 ms in CTRL, P = 0.057) (Fig. 5F). Such behavior of the forward mode Na⁺/Ca²⁺ exchanger could be due to increased intracellular [Na⁺] and would lead to incomplete removal of the Ca²⁺ released from the SR out of the cell. We indeed observed that [Ca²⁺], at the end of the caffeine pulse remained significantly above baseline in the presence of KB (26.8 ± 5.3 nM, n_cells = 10) and returned to baseline only after removal of caffeine, whereas this was not the case in control. With incomplete removal, the integral of the Na⁺/Ca²⁺ exchanger current is no longer reliably reflecting SR content, explaining why we failed to observe a significant increase in the integrated NCX current.

Increased [Na⁺]i and incomplete removal of Ca²⁺ are also expected to lead to fast reloading of the SR after removal of caffeine. This was tested by applying a second pulse of caffeine 10 s after washout of the first application, with the membrane potential held constant at −50 mV. As illustrated in Fig. 6A, a second caffeine application in CTRL conditions did not evoke a second Ca²⁺ release (no transient increase of [Ca²⁺]i, only an increase of baseline [Ca²⁺]i by less than 100 nM). However, with KB, a second release of Ca²⁺ from the SR was observed as a [Ca²⁺]i transient during the second caffeine pulse, consistent with incomplete removal during the first application, and fast reloading of the SR (peak of the [Ca²⁺]i transient during the second pulse of caffeine was 316.6 ± 49.7 versus 156.15 ± 25.7 nM for the maximal value of [Ca²⁺]i, during caffeine application in CTRL, n_cells = 10, P < 0.01, Fig. 6B).

The Effect of KB on the Na⁺/Ca²⁺ Exchanger Is Mediated by an Increase in [Na⁺]i. If the reduction in forward mode Na⁺/Ca²⁺ exchange is due to an increase in [Na⁺]i, we expect to see an increase in reverse-mode Na⁺/Ca²⁺ exchange. Therefore, we measured the outward current during a step to +40 mV and blocked by 2.5 mM NiCl₂ before and after KB application. This nickel-sensitive current was indeed significantly increased after KB application (0.65 ± 0.14 versus 0.37 ± 0.09 pA/pF in CTRL, n_cells = 7, P < 0.01, Fig. 7A).

To further elucidate whether there is indeed an increase in [Na⁺]i, we measured the Na/K-ATPase current that is directly related to changes in [Na⁺]i, if membrane potential is constant (for review, see Glitsch, 2001; Verdonck et al., 2003). Therefore, we measured the current suppressed by 10 μM dihydrouobain, at −50 mV, following a train of pulses from −70 to +10 mV. At this potential, I_NaK is small (0.17 ± 0.02 pA/pF), but it was more than 2-fold increased with KB (0.38 ± 0.06 pA/pF, n_cells = 5, P < 0.05, Fig. 7B).

Discussion

KB130015, at a dose of 10 μM that significantly slows I_Na inactivation, increases the amplitude of the Ca²⁺ transient...
Mechanisms Underlying the Increase in [Ca\(^{2+}\)]\(_i\), with KB. In the presence of a reduction of I\(_{Ca,L}\), the first explanation for the increase in the amplitude of the [Ca\(^{2+}\)]\(_i\) transient with KB is the increase in SR Ca\(^{2+}\) content resulting from the higher intracellular [Na\(^+\)]. We also have to consider, however, that with the increase in [Na\(^+\)], enhanced influx of Ca\(^{2+}\) via reverse-mode Na\(^+\)/Ca\(^{2+}\) exchange during depolarization can contribute to this increase. We have found previously that reverse mode per se is a very inefficient trigger for Ca\(^{2+}\) release and that the Ca\(^{2+}\) release seen with the Na\(^+\) current most likely represents spurious activation of Ca\(^{2+}\) channels rather than activation of release channels via reverse mode (Sipido et al., 1995, 1997). This view has been supported by others (for review, see Bers, 2002), although there have been reports that Ca\(^{2+}\) release triggered by reverse-mode Na\(^+\)/Ca\(^{2+}\) exchange following Na\(^+\) influx via the channel had a specific profile (Lipp and Niggli, 1994). Others have argued that Ca\(^{2+}\) influx via Na\(^+\)/Ca\(^{2+}\) exchange can modulate and reinforce the trigger function of I\(_{Ca,L}\) (Litwin et al., 1998). In Fig. 3B, we saw that the increase in amplitude of the [Ca\(^{2+}\)]\(_i\), transient activated by I\(_{Ca,L}\) alone during the step from −50 to +10 mV is less than the increase in the [Ca\(^{2+}\)]\(_i\), transient during the action potential or the voltage-clamp step from −70 to +10 mV that activated both I\(_{Na}\) and I\(_{Ca,L}\). This can be taken to support the idea that Ca\(^{2+}\) influx associated with the Na\(^+\) current contributes to the enhanced [Ca\(^{2+}\)]\(_i\), transient with KB. It is not absolute proof, however, because the release with the step from −70 to −50 mV has partially depleted the sarcoplasmic reticulum. Another argument in favor of a role for reverse mode is the prolongation of the time to peak [Ca\(^{2+}\)]\(_i\), during action potentials and single square voltage steps from −70 to +10 mV but not in the steps from −50 to +10 mV. This is compatible with the presence of a slower process triggering Ca\(^{2+}\) release or contributing direct Ca\(^{2+}\) influx in the presence of Na\(^+\) current only. This could be reverse-mode Na\(^+\)/Ca\(^{2+}\) exchange, in particular in the presence of a reduced I\(_{Ca,L}\).

Another argument in favor of increased Ca\(^{2+}\) influx via reverse-mode Na\(^+\)/Ca\(^{2+}\) exchange during depolarization comes from examining the Ca\(^{2+}\) flux balance. With a depolarizing step, Ca\(^{2+}\) extrusion via Na\(^+\)/Ca\(^{2+}\) exchange on repolarization should match the Ca\(^{2+}\) influx during the step (Bridge et al., 1990; Trafford et al., 1997; Bers, 2002). We calculated the integral of the Na\(^+\)/Ca\(^{2+}\) exchange forward mode on repolarization after the single depolarizing pulse and found that it was unchanged with KB (data not shown). However, because Ca\(^{2+}\) influx via I\(_{Ca,L}\) is reduced, we expect to find a reduced value; thus, this observation can support the concept that there has been additional Ca\(^{2+}\) influx via reverse-mode Na\(^+\)/Ca\(^{2+}\) exchange.

Is KB a Useful Inotropic and/or Antiarrhythmic Agent? The finding of a moderate increase in Ca\(^{2+}\) release without spontaneous Ca\(^{2+}\) oscillations can be taken as favorable in comparison with other Na\(^+\) channel openers such as DPI 201-106, BDF 9148, and BDF 9198 (for review, see Fleisch and Erdmann, 2001). To some extent, this must be seen as a dose effect. Indeed, in a few experiments, we used KB at 50 \(\mu\)M and saw a larger increase in [Ca\(^{2+}\)]\(_i\) and [Na\(^+\)], with arrhythmogenic effects (data not shown). On the other hand, there is a genuine difference between KB and these substances, namely the presence of Ca\(^{2+}\) channel blockade that limits total Ca\(^{2+}\) influx and protects against excessive Ca\(^{2+}\) loading. Caution remains, however, in the failing heart because [Na\(^+\)], is already elevated, and a further increase in [Na\(^+\)], might more rapidly lead to Ca\(^{2+}\) overload.

Thus, although the effects on systolic Ca\(^{2+}\) can be overall considered rather favorable, the effects on diastolic Ca\(^{2+}\) are less favorable. Indeed, the total duration of the [Ca\(^{2+}\)]\(_i\), transient is somewhat prolonged and diastolic [Ca\(^{2+}\)]\(_i\), levels are slightly increased, factors that both will likely reduce diastolic filling in the intact heart. This is particularly relevant for patients with a compromised diastolic function as often observed in heart failure (Kass et al., 2004).

In contrast to classical Na\(^+\) channel openers, KB does not markedly prolong the action potential; thus, its potential antiarrhythmic activity cannot be related to a typical class III effect. However, there are some unique properties that...
may help to explain the observed protection (Mubagwa et al., 2003). Triggered early and delayed afterdepolarizations are important mechanisms underlying ventricular arrhythmias in heart failure; KB may partially reduce the likelihood of both these mechanisms.

Early afterdepolarizations have been ascribed to reactivation of I_{Ca,L} (January and Riddle, 1989; Zeng and Rudy, 1995); thus, a partial block of I_{Ca,L} may be a favorable property of KB. It could be argued that Na\(^{+}\)/Ca\(^{2+}\) window currents could provide EADs with KB, as seen with anemone toxin II (Boutjdir et al., 1994) or veratridine (Verdonck et al., 1991). A difference with these substances is, however, that they induce a large persistent current that is again not seen with KB. Inward Na\(^{+}\)/Ca\(^{2+}\) exchange current in the setting of enhanced Ca\(^{2+}\) loading can provide the conditioning current to allow the EAD (Zeng and Rudy, 1995; Volders et al., 1997; Wehrens et al., 2000). As we have seen, the inward Na\(^{+}\)/Ca\(^{2+}\) exchange current is not increased but rather reduced with KB, probably because of the higher intracellular Na\(^{+}\) and leftward shift of the reversal potential. This same mechanism, i.e., a reduced forward Na\(^{+}\)/Ca\(^{2+}\) exchange current, can also help to reduce the likelihood and amplitude of delayed afterdepolarizations if spontaneous Ca\(^{2+}\) release would occur. Lastly, the higher amplitude of the outward sodium/potassium pump current may exert a stabilizing influence on the resting membrane potential. As elegantly demonstrated by Pogwizd et al. (2001), a reduction in outward I_{K1} at the resting membrane potential, as seen in the rabbit with heart failure, is an important factor in facilitating delayed afterdepolarizations. KB would counteract enhanced excitability by providing additional outward current at the resting membrane potential.

Despite its delaying of Na\(^{+}\) channel inactivation, KB does not prolong the action potential. The reduction of I_{Ca,L} is one element that contributes, as discussed before (Mubagwa et al., 2003). In the present study, we identify another, namely the larger sodium/potassium pump current. The lack of an increase in action potential duration is favorable to reduce EADs and may also be favorable for the diastolic function.
Conclusions

Slowing of the Na+ channel inactivation by KB130015 leads to an increase in [Na+], and consequently in cellular Ca2+ load. This effect is partially offset by a decrease in ICa-L, resulting in a mild inotropic effect without signs of Ca2+ overload and related arrhythmias. Activation of the sodium/potassium pump current by the increased [Na+], may contribute to an antiarrhythmic effect.

References


Fig. 7. KB increases reverse-mode Na+/Ca2+ exchange through increased [Na+]. A, individual and averaged data of the amplitude of nickel-sensitive outward current during a step to +40 mV before and after KB superfusion. n = 7; **, P < 0.01. B, pooled data of the amplitude of the outward Ca2+-sensitive current at −50 mV, recorded after a series of depolarizing pulses from −70 to +10 mV. V_rest = 5; *, P < 0.05.


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