Beneficial Effect of Calcium Treatment for Hyperkalemia Is Not Due to "Membrane Stabilization"

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OBJECTIVES: Hyperkalemia is a common life-threatening condition causing severe electrophysiologic derangements and arrhythmias. The beneficial effects of calcium (Ca^{2+}) treatment for hyperkalemia have been attributed to "membrane stabilization," by restoration of resting membrane potential (RMP). However, the underlying mechanisms remain poorly understood. Our objective was to investigate the mechanisms underlying adverse electrophysiologic effects of hyperkalemia and the therapeutic effects of Ca^{2+} treatment.

DESIGN: Controlled experimental trial.

SETTING: Laboratory investigation.

SUBJECTS: Canine myocytes and tissue preparations.

INTERVENTIONS AND MEASUREMENTS: Optical action potentials and volume averaged electrocardiograms were recorded from the transmural wall of ventricular wedge preparations ($n = 7$) at baseline (4 mM potassium), hyperkalemia (8–12 mM), and hyperkalemia + Ca^{2+} (3.6 mM). Isolated myocytes were studied during hyperkalemia (8 mM) and after Ca^{2+} treatment (6 mM) to determine cellular RMP.

MAIN RESULTS: Hyperkalemia markedly slowed conduction velocity (CV, by 67% ± 7%; $p < 0.001$) and homogeneously shortened action potential duration (APD, by 20% ± 10%; $p < 0.002$). In all preparations, this resulted in QRS widening and the "sine wave" pattern observed in severe hyperkalemia. Ca^{2+} treatment restored CV (increase by 44% ± 18%; $p < 0.02$), resulting in narrowing of the QRS and normalization of the electrocardiogram, but did not restore APD. RMP was significantly elevated by hyperkalemia; however, it was not restored with Ca^{2+} treatment suggesting a mechanism unrelated to "membrane stabilization." In addition, the effect of Ca^{2+} was attenuated during L-type Ca^{2+} channel blockade, suggesting a mechanism related to Ca^{2+}-dependent (rather than normally sodium-dependent) conduction.

CONCLUSIONS: These data suggest that Ca^{2+} treatment for hyperkalemia restores conduction through Ca^{2+}-dependent propagation, rather than restoration of membrane potential or "membrane stabilization." Our findings provide a mechanistic rationale for Ca^{2+} treatment when hyperkalemia produces abnormalities of conduction (i.e., QRS prolongation).

KEYWORDS: calcium; cardiac conduction; electrocardiography; hyperkalemia; resting membrane potential

Hyperkalemia is a common electrolyte abnormality seen in critical care settings and is potentially life threatening (1, 2). Elevated extracellular potassium concentration can result in well-described electrocardiogram (ECG) changes. Generally, during mild to moderate hyperkalemia, shortened, peaked T waves are observed, indicating an effect on ventricular repolarization.

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KEY POINTS

Question: To determine the mechanisms underlying adverse electrophysiologic effects of hyperkalemia and the therapeutic effects of calcium (Ca^{2+}) to better inform treatment of hyperkalemia.

Findings: These data demonstrate that Ca^{2+} treatment for hyperkalemia restores conduction by promoting calcium inward current-dependent propagation, rather than restoration of resting membrane potential or "membrane stabilization."

Meaning: This study provides a mechanistic rationale for Ca^{2+} treatment when hyperkalemia produces abnormalities of conduction (i.e., QRS prolongation).

During more severe hyperkalemia, conduction slowing occurs, producing a widened QRS interval on the ECG, which when severe, eventually merges with the T wave, creating a sine wave pattern. Arrhythmias attributable to hyperkalemia include bradycardias, conduction block, and ventricular tachycardia and fibrillation, resulting in cardiovascular dysfunction and death (1, 3, 4). However, the effects of elevated potassium on arrhythmia pathophysiology (i.e., substrates) are not fully understood (1). Previously it has been shown that hyperkalemia heterogeneously alters the action potential (AP) duration (APD) in epicardial and endocardial myocytes (5). Heterogeneities in APD increase spatial dispersion of repolarization (DOR), which can lead to the development of reentrant arrhythmias, ventricular tachycardia/fibrillation, and sudden cardiac death. Hyperkalemia also elevates cellular resting membrane potential (RMP), which decreases cellular fast sodium channel function and excitability, resulting in slowed conduction and block, a prerequisite for the development of reentrant arrhythmias (1, 6, 7).

IV calcium (Ca^{2+}) is a common treatment for the adverse cardiac effects of hyperkalemia, which have been attributed to its "membrane stabilizing effects," a vague term frequently used, and typically attributed to normalization of RMP (8–11). However, despite decades of everyday use, the mechanism by which Ca^{2+} improves cardiac conduction and function in hyperkalemia remains incompletely understood (1). Consequently, there is significant heterogeneity in the clinical threshold for treating hyperkalemia with Ca^{2+} (12). Whether IV Ca^{2+} is protective by improving hyperkalemic abnormalities in only conduction, repolarization, or both is poorly understood. Furthermore, improved understanding of these effects would help guide clinicians in appropriate use of Ca^{2+} treatment in hyperkalemia.

In this study we use ex vivo tissue and in vitro cellular approaches to determine the electrophysiologic basis for the beneficial effect of Ca^{2+} on the ECG and arrhythmia substrates. Given that Ca^{2+} improves conduction in conditions when cardiomyocyte RMP is markedly elevated, such as in acute ischemia (7), we hypothesized that the beneficial effects of Ca^{2+} treatment during hyperkalemia are based on direct preservation of cardiac conduction, but not effects on membrane stabilization or cardiac repolarization.

MATERIALS AND METHODS

All experiments were carried out in accordance with Public Health Service guidelines for the care and use of laboratory animals and approved by our institutional animal care and use committee (Board name: CWRU IACUC, Study Number: Protocol 070161, Study title: Cell Repolarization, Alternans, and Arrhythmogenesis, Approval Date: January 18, 2008, Animal Welfare Assurance No. A3145-01). Eight adult, male, random source, mongrel dogs were used in this study. To minimize animal pain and suffering, these experiments were conducted on tissue collected by organ harvest only after deep surgical anesthesia was established with pentobarbital. The canine species was chosen because of the well-known electrophysiologic similarities to humans and the canine wedge preparation is an established model for the human ECG (13). Male dogs were specifically used to eliminate variability induced by sex-related heterogeneities in repolarization and sensitivity to potassium (14). We used data from our prior work in the canine wedge preparation, which examined conduction and repolarization heterogeneities underlying arrhythmias, as the basis for a sample size calculation used to estimate the number experiments and dogs required (please see Animal Research: Reporting of In Vivo Experiments guidelines for further detail) (15). We used two complimentary approaches: 1) the canine wedge preparation which allowed examination of the effects of potassium and calcium on conduction, repolarization, and the ECG in a well-established tissue model and 2) isolated canine myocytes, which allows

for precise determination of cellular RMP under a variety of conditions but does not allow determination of cell-to-cell conduction.

Optical Mapping in the Canine Wedge Preparation

We have previously described our methods for optical mapping in the canine wedge preparation (15–18). Briefly, the intact heart was rapidly excised by right lateral thoracotomy performed under pentobarbital (50 mg/mL IV) anesthesia. Transmural wedges of left ventricular myocardium were isolated and the branch of the corresponding coronary artery was cannulated and perfused with Tyrode's solution (140 mM sodium chloride [NaCl], 4.0 mM potassium chloride [KCl], 1.8 mM Ca^{2+} chloride, 5.5 mM dextrose, 0.5 mM magnesium sulfate [$MgSo_4$], 0.9 mM monosodium phosphate, 10 mM 4-(2-hydroxyethyl)-1-piperazineethansulfonic acid [HEPES], sodium hydroxide [NaOH] titrated to a pH 7.41, and oxygenated with 100% oxygen). The wedge was then placed in the chamber with the transmural surface against the glass imaging plate and perfused with a voltage sensitive dye di-4-aminonaphthenyl-pyridinum-propylsulfonate (8 μM). APs were recorded from 256 sites simultaneously with high spatial (0.89–1.1 mm), temporal (1 ms), and voltage (0.5 mv) resolution. Blebbistatin (6 μM) was used to eliminate motion artifact. The imaging chamber was insulated and temperature closely regulated by an insulated water circuit and measured using a digital temperature probe (Omega Engineering Inc., Norwalk, CT) in the water bath, allowing for temperature precision of ±0.1°C (15). Measurements were made at baseline (4 mM) potassium concentration $[K^+]o$ in the Tyrode's solution, and then increased to 8 mM, and 12 mM $[K^+]o$. Ca^{2+} was added to the 12 mM $[K^+]o$ solution to create a Ca^{2+} concentration that was 2× the normal (3.6 mM). This is consistent with expected rise of Ca^{2+} observed after 20 mg/kg of IV Ca^{2+} (19).

Groups and Methods of Measurement. APD was measured in any one transmural layer by averaging 5 epicardial, mid-myocardial, and endocardial cell APDs, respectively. Transmural cell types were defined by previously validated anatomic and functional criterion (18, 20). DOR was defined as the difference between the APD of the longest and shortest cell type. Conduction velocity (CV) and maximal repolarization gradients (RGs) were determined by a previously validated vector analysis technique (21, 22). Briefly, CV and RG vectors between nearby sites of the 256 site array were calculated and averaged across the maximal direction of depolarization (CV) and repolarization (RG) to obtain the average CV and RG per wedge. Measurements were always made during endocardial pacing (to reproduce normal endocardial to epicardial activation in the heart).

Isolated Cardiomyocyte Studies

Patch-Clamp Recordings. The amphotericin perforated patch technique was used to obtain whole-cell recordings of membrane voltage under current-clamp conditions as described previously (23). Briefly, the cells were bathed in a chamber continuously perfused with Tyrode's solution composed of (mmol/L) NaCl 137, KCl 5.4, calcium chloride ($CaCl_2$) 2.0, $MgSO_4$ 1.0, glucose 10, HEPES 10, and pH to 7.35 with NaOH. Patch pipettes were pulled from borosilicate capillary glass and lightly fire-polished to resistance 0.9–1.5 mol/LΩ when filled with electrode solution composed of (mmol/L) aspartic acid 120, KCl 20, NaCl 10, magnesium chloride, HEPES 5, 240 μg/mL of amphotericin B (Sigma, St Louis, Mo), and pH 7.3. A gigaseal was rapidly formed. Typically, 10 minutes later, amphotericin pores lowered the resistance sufficiently to current-clamp the cells. Myocytes were paced using a 1.5–2 diastolic threshold and a 5-ms current pulse. Experiments were performed at 30°C. Command and data acquisition were operated with an Axopatch 200B patch-clamp amplifier controlled by a personal computer using a Digidata 1200 acquisition board driven by pCLAMP 7.0 software (Axon Instruments, Foster City, CA). Cells were superfused with different $[K^+]o$ concentration at 4 and 8 mM KCl with and without additional CaCl to increase Ca^{2+} concentration to 6 mM, with and without addition of verapamil (10 uM) or tetrodotoxin (120 uM). Myocytes were stimulated at a baseline stimulation rate of 150 beats/min. After a period of stimulation to establish steady state, measurements were made for the subsequent 20 beats/min. APD was measured at 90% repolarization.

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics 24 (IBM Corp, Armonk, NY) and Excel (Microsoft, Redmond, WA). One-way analysis of variance was used to compare differences mean APD, CV, and DOR across wedge experiments

under different potassium concentrations and least square differences post hoc test was applied to test specific means. Student *t* tests (two-way, unpaired) were used to analyze the differences in RMP and APD under hyperkalemic conditions in isolated myocytes. All mean data are represented with the value and SD. Previous power analyses suggested $n = 5$ per group to detect a significant difference in DOR in the canine wedge with a power of 0.8 and an error of 0.05 (15).

RESULTS

The Effect of Hyperkalemia on Cardiac Conduction and Repolarization

Figure 1 shows the effect of hyperkalemia on the ECG, myocardial conduction, and repolarization. Hyperkalemia produced marked conduction slowing, as evidenced by: 1) QRS widening of the ECG (upper row, ECG 4 vs. 12 mM $[K^+]o$), 2) increase in time between depolarization of the early activated endocardial AP and late activated epicardial AP (middle rows, 4 vs. 12 mM $[K^+]o$, red hatched boxes), and 3) marked transmural conduction slowing and block, in which hyperkalemia (12 mM) shows significant crowding of activation time isochrones compared with baseline (4 mM, lower row). Of note, there is also conduction block observed in the subepicardium (solid line) during hyperkalemia. Summary data of CV over all experiments is shown in **Figure 2**. Hyperkalemia clearly slowed transmural conduction (by 67% ± 7%; $p < 0.002$ at 12 mM $[K^+]o$).

As expected, hyperkalemia also shortened APD. Shown in Figure 1, middle rows, are endocardial and epicardial APs, where there is moderate shortening of endocardial and epicardial APs, with an average 20% ± 10% decrease ($p < 0.002$). This observation was confirmed in isolated myocytes, where APD shortening was observed at 8 mM $[K^+]o$ (average 47% decrease; $p < 0.002$; **Fig. 3*A***, control vs. high K tracings). As AP heterogeneity and increased DOR can be a potent substrate for arrhythmias, we next examined whether hyperkalemia increased heterogeneity of APD in the wedge preparation. **Figure 4** shows average APD from endocardial and epicardial cell types across the transmural wall. Similar APD shortening during 12 mM $[K^+]o$ was observed between the different cell types, demonstrating there was no change in transmural

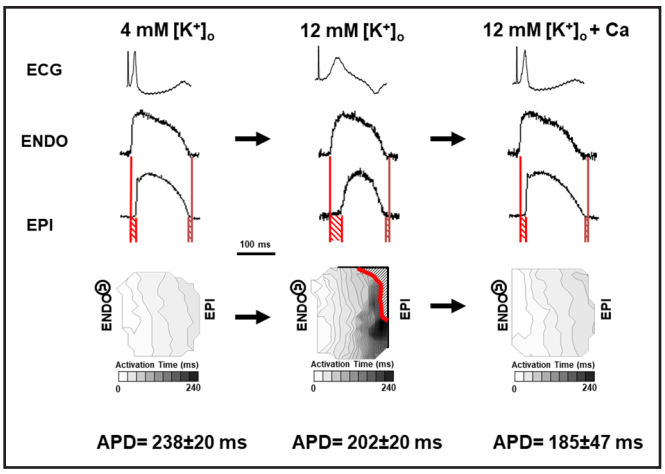

Figure 1. Hyperkalemia slows conduction, which is rescued by calcium treatment. **Top row** shows the effect of hyperkalemia and calcium (Ca^{2+}) treatment on the electrocardiogram (ECG). During hyperkalemia, a wide QRS showing a sine wave pattern is observed, which is normalized by Ca^{2+} treatment. **Second row** shows endocardial (ENDO) and epicardial (EPI) action potentials (APs) at baseline, during hyperkalemia, and Ca^{2+} treatment. At baseline, transmural conduction time (difference between activation time of early activated ENDO and late activated EPI APs is relatively short [*red hatched lines*]). Difference in repolarization times (a marker of dispersion of repolarization [DOR]) is also shown (*orange hatched lines*). During hyperkalemia, marked conduction slowing occurs, with small prolongations in DOR. During Ca^{2+} treatment, conduction time normalizes. Representative isochronal contour maps of conduction time (**third row**) are also shown under the three conditions. Earliest conduction is in *white*, while later conduction or repolarization is shown by *darker colors*. Note that with 12 mM $[K^+]$, there is marked conduction slowing, as indicated by crowding of isochrones and conduction block occurs in the subepicardium (*dark line*). With Ca^{2+} treatment, conduction velocity normalizes, and conduction block is prevented. Summary data for mean action potential duration (APD) under each condition is also shown at the **bottom row**.

DOR. Taken together, these data show that although APD is shortened during hyperkalemia, APD heterogeneity does not increase and therefore would not necessarily contribute to increased arrhythmia risk.

The Effect of Ca^{2+} on Hyperkalemia-Induced Conduction Slowing and Action Potential Duration

Figure 1, far right column, shows normalization of the ECG (upper row), when hyperkalemia is treated with Ca^{2+} when compared with hyperkalemia alone (center column). This is associated with normalization of CV, demonstrated by decreased time between

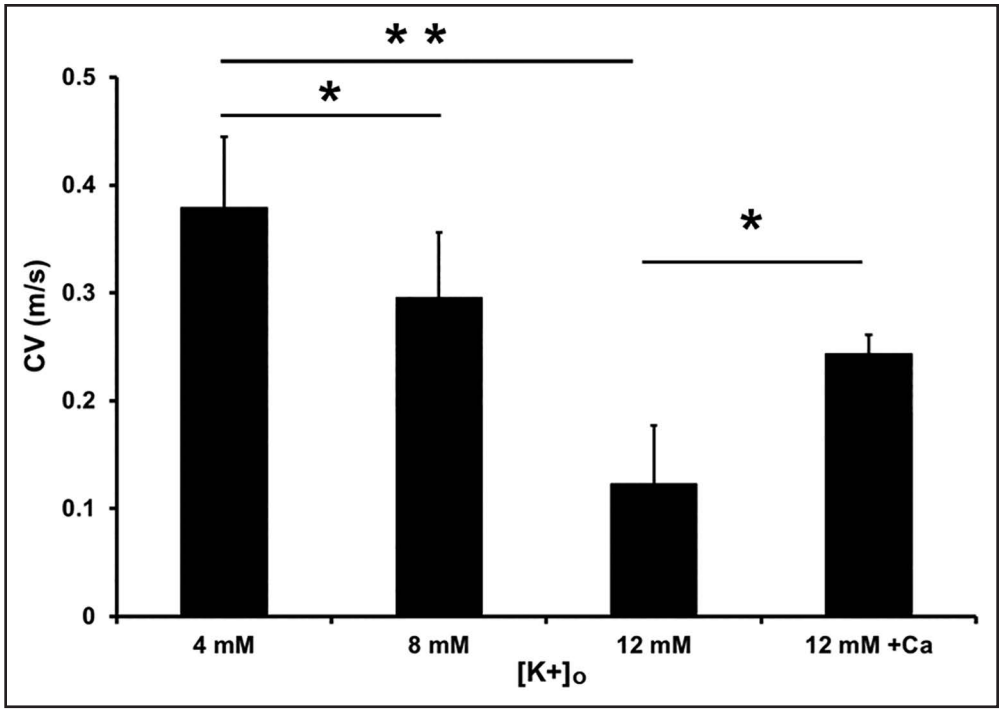

Figure 2. Summary data of conduction velocity (CV) over all experiments is shown. The greatest CV slowing from baseline (4 mM) was observed between 8 and 12 mM [K^+]. Calcium (Ca^{2+}) improved conduction, which is expected to be antiarrhythmic ($n = 7$ preparations under all conditions except 8 mM, $n = 5$, $^*p < 0.02$, $^{**}p < 0.001$).

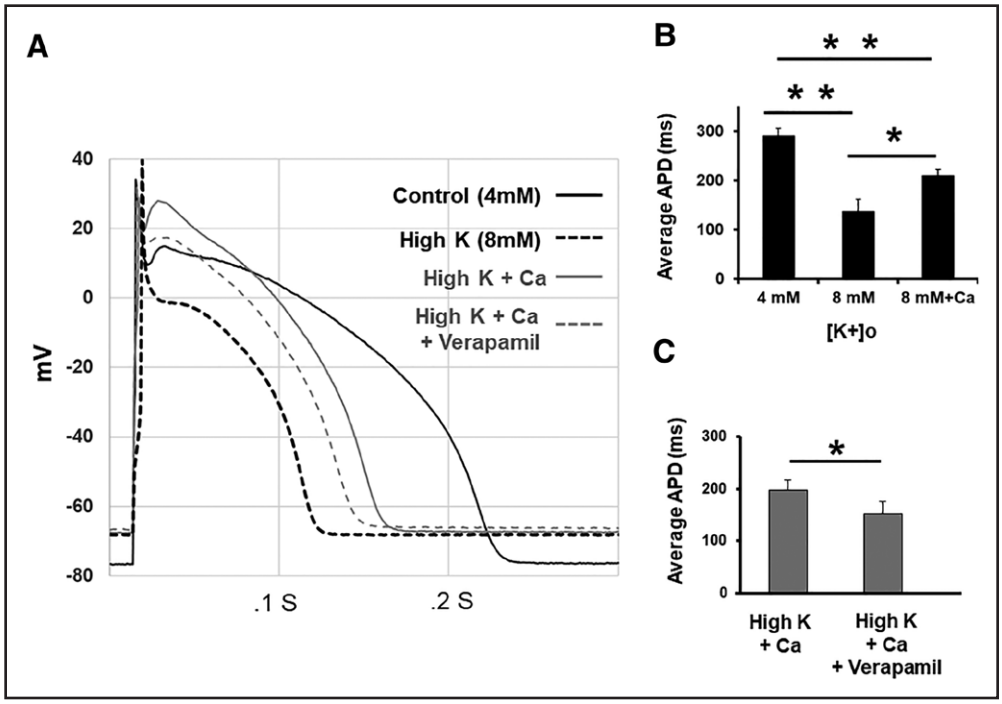

Figure 3. Effect of hyperkalemia and experimental conditions on the action potential (AP). **A**, Shown are tracings of cellular APs recorded at baseline (*black*), hyperkalemia (8 mM, *gray*), and after calcium (Ca^{2+}) treatment (*stippled*) from isolated cardiomyocytes. Marked AP shortening occurs with hyperkalemia, which is improved with Ca^{2+} treatment ($^*p < 0.05$, ** $p < 0.01$, $n = 7$). **B**, Summary data for action potential duration (APD) under the three conditions is shown. **C**, Summary data for high K + Ca condition, with and without verapamil, are shown.

endocardial and epicardial activation (middle rows), as well as normalization of CV (lower row), as compared with hyperkalemia alone (center column). Note that although conduction block is observed at 12 mM, this is no longer evident in the presence of Ca^{2+} treatment. Summary data over all experiments (Fig. 2) demonstrates attenuation of hyperkalemia-induced CV slowing by Ca^{2+} and this effect was dose dependent (**Supplemental Fig. 1**, http://links.lww.com/CCM/H567). In the wedge preparation, we did not observe any significant changes in APD and DOR with Ca^{2+} treatment during hyperkalemia (Fig. 4). In contrast to what was observed in tissue preparations, in isolated myocytes, the addition of Ca^{2+} partially mitigated hyperkalemia-induced APD shortening (high K vs. high K + Ca^{2+} and summary data in **Fig. 3*B***).

Mechanisms Underlying Restoration of CV by Ca^{2+} During Hyperkalemia

We next examined the effect of hyperkalemia and Ca^{2+} on RMP. As this cannot be evaluated using optical mapping in tissue, this was tested in isolated myocytes. As shown in **Figure 5*A***, as expected, hyperkalemia (8 mM) significantly increased RMP.

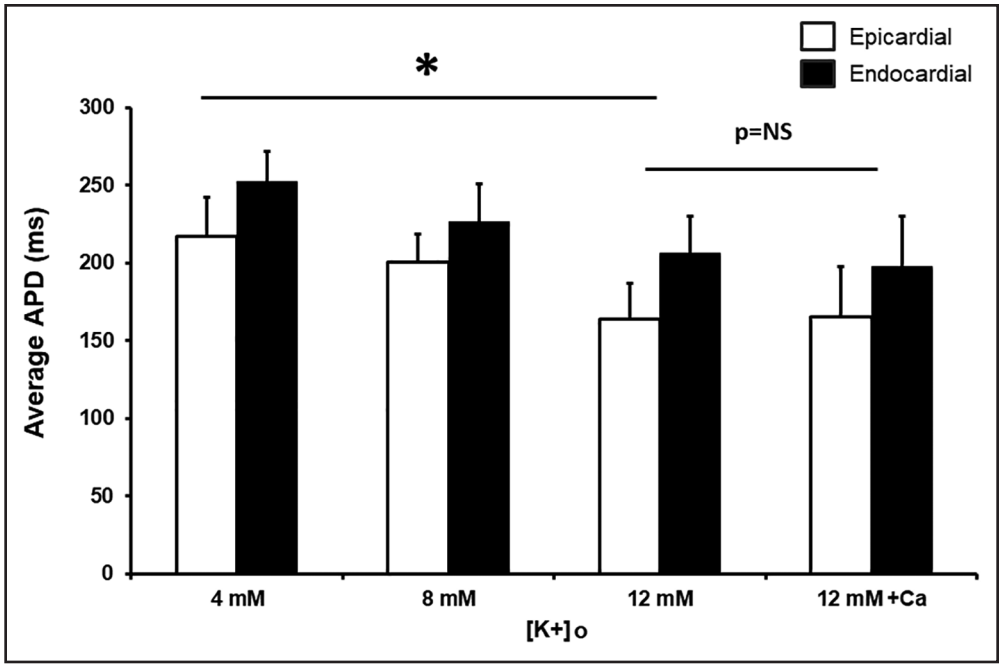

Figure 4. Effect of hyperkalemia and Ca+ on transmural action potential duration (APD) and dispersion of repolarization (DOR). Summary data of APD as determined in the canine wedge preparation is shown. Compared with baseline (4 mM [K+]) at high potassium, APD is shortened, but DOR is not significantly (NS) affected (difference between epicardial and endocardial APD) even in presence of calcium treatment (*$p < 0.05$).

Interestingly, treatment with Ca^{2+} had no effect on RMP, which was reproducible over all experiments (summary data in **Fig. 5*B***). This strongly suggests that the mitigation of CV we observed was not secondary to an effect on RMP or "membrane stabilization."

We next investigated a potential alternative mechanism for improvement in CV secondary to Ca^{2+} treatment, based on calcium inward current-dependent propagation (7, 24) using a pharmacological approach. In **Figure 6*A*** are representative isochrone maps from a wedge preparation at baseline (4 mM $[K^+]$o, upper row, far left), after induction of hyperkalemia (12 mM $[K^+]$o, no treatment), treatment with Ca^{2+} and then Ca^{2+} treatment in the presence of the L-type Ca^{2+} channel blocker verapamil (20 uM). As expected, during hyperkalemia, marked conduction slowing, as noted by crowding of isochrones, and conduction block (solid line), were observed. In the presence of Ca^{2+}, conduction slowing is mitigated, and block is prevented. However, in the presence of verapamil, conduction slowing and block returns. Summary data shows that verapamil prevents CV improvements by Ca^{2+} treatment during hyperkalemia (**Fig. 6*B***). Taken together, this suggests that during hyperkalemia, L-type Ca^{2+} current augmentation by Ca^{2+} treatment is involved in the mechanism of increased CV. To ensure verapamil had no effects on RMP, this was investigated in single cell studies, which showed no effect of verapamil on hyperkalemia-induced RMP elevation during Ca^{2+} treatment (**Fig. 5**, *A* and ***C***). In addition, the presence of verapamil mitigated the partial restoration of hyperkalemia-induced APD shortening by Ca^{2+} (Fig. 3*A*, high K + Ca^{2+} vs. high K + Ca^{2+} plus verapamil tracings and **Fig. 3*C***). This suggests that increased L-type Ca^{2+} current secondary to Ca^{2+} administration is also responsible for the APD prolongation we observed in isolated myocytes. Finally, to investigate whether the effect of Ca^{2+} treatment during hyperkalemia was mediated by an effect on the sodium channel, in isolated myocytes, we conducted studies in the presence of the sodium channel blocker tetrodotoxin (120 mM). No attenuation of the effect of Ca^{2+} on RMP or APD was seen in isolated myocytes in the presence of tetrodotoxin, $n = 7$ (data shown in **Supplemental Fig. 2**, http://links.lww.com/CCM/H567), further suggesting that the mechanism by which Ca^{2+} restores CV during hyperkalemia was not related to enhanced sodium channel excitability. We observed differences between tetrodotoxin and verapamil on APD under conditions of hyperkalemia and Ca^{2+} treatment, where verapamil, but not tetrodotoxin, shortened APD (Supplemental Fig. 2, *A* and *C*, http://links.lww.com/CCM/H567), suggesting a more preferential effect on L-type Ca^{2+} by verapamil in our experiments.

DISCUSSION

High potassium significantly slows conduction and shortens APD, creating arrhythmogenic substrates. Ca^{2+} treatment results in improved conduction, normalizing the ECG. Our data are consistent with known

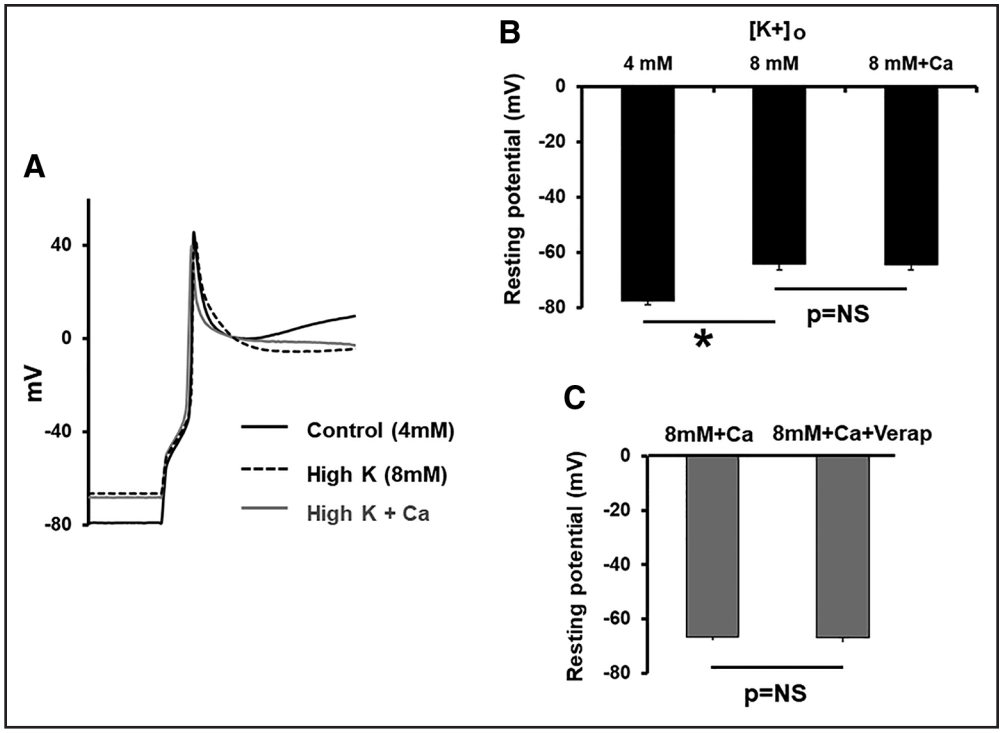

Figure 5. Effect of hyperkalemia and Ca_+ resting membrane potential (RMP). **A**, Shown are RMP and phase 0 depolarization of representative action potentials in control (*black*), hyperkalemia (8 mM potassium, *black stippled*) and hyperkalemia with calcium (Ca^{2+}) treatment (*gray*). Note that although RMP rises during hyperkalemia, it is not affected by Ca^{2+} treatment. **B**, Summary data for RMP is shown (*$p < 0.01$, $n = 7$). **C**, Summary data for RMP during hyperkalemia and Ca_{2+} treatment, with and without verapamil (Verap), is shown. NS = not significant.

effects of hyperkalemia on RMP, whereby as hyperkalemia progresses, RMP increases. Also consistent with clinical and experimental observations, we observed improved CV with Ca^{2+} treatment (1, 7, 9). However, we observed no improvement in RMP with Ca^{2+} treatment, strongly suggesting that the effect we observed is not related to "membrane stabilization." Furthermore, these data provide a mechanistic rationale for clinical use of Ca^{2+} treatment for hyperkalemia when the ECG reveals slow conduction (i.e., QRS prolongation).

Effect of Hyperkalemia and Calcium on Conduction Velocity and "Membrane Stabilization"

As hyperkalemia progresses, the initial rise in RMP decreases the threshold for sodium current activation thereby increasing conduction. However, a further rise in RMP will decrease sodium channel availability, thereby decreasing conduction. This balance is likely why an increase in CV is observed at lower ranges of hyperkalemia, then slowed conduction at more severe hyperkalemia (1, 6). The evidence of a protective effect of Ca^{2+} on cardiac excitability and "membrane stabilization" is based on early observations by Winkler et al (25) in 1939 where Ca^{2+} prevented cardiac standstill during hyperkalemia and by additional experimental observations that at markedly high Ca^{2+} concentrations, Ca^{2+} itself elevates RMP (6, 8, 9, 26). However, in the current study, at physiologically relevant calcium concentrations in isolated myocytes, we did not observe a change in RMP by increasing Ca^{2+} (19). Nonetheless, we observed that Ca^{2+} administration restored CV and normalized the ECG. Furthermore, our data demonstrating that L-type Ca^{2+} channel blockade prevents improvements in CV by Ca^{2+} treatment during hyperkalemia (without a change in RMP), suggests a mechanism related to Ca^{2+} mediated conduction, rather than an effect on RMP. Ca^{2+} mediated conduction has been described in ischemia and other conditions with elevated RMP (7, 24, 27) and involves maintenance of impulse propagation by cellular excitability through L-type Ca^{2+} current. An additional, potentially complimentary, mechanism is that changes in extracellular Ca^{2+} concentrations may promote ephaptic coupling, thereby improving CV. Ephaptic coupling, refers to the activation of voltage gated ion channels and impulse propagation by extracellular potential gradients in the perinexal space between adjacent myocytes. Specifically, regarding hyperkalemia, increased Ca^{2+} concentrations have been shown to decrease the width of the perinexus, which is expected to enhance ephaptic coupling and improve conduction (28, 29).

Effects of Hyperkalemia and Calcium on Repolarization and the T Wave

As expected, hyperkalemia shortened APD, which has been attributed to the changes in peaked and narrowed

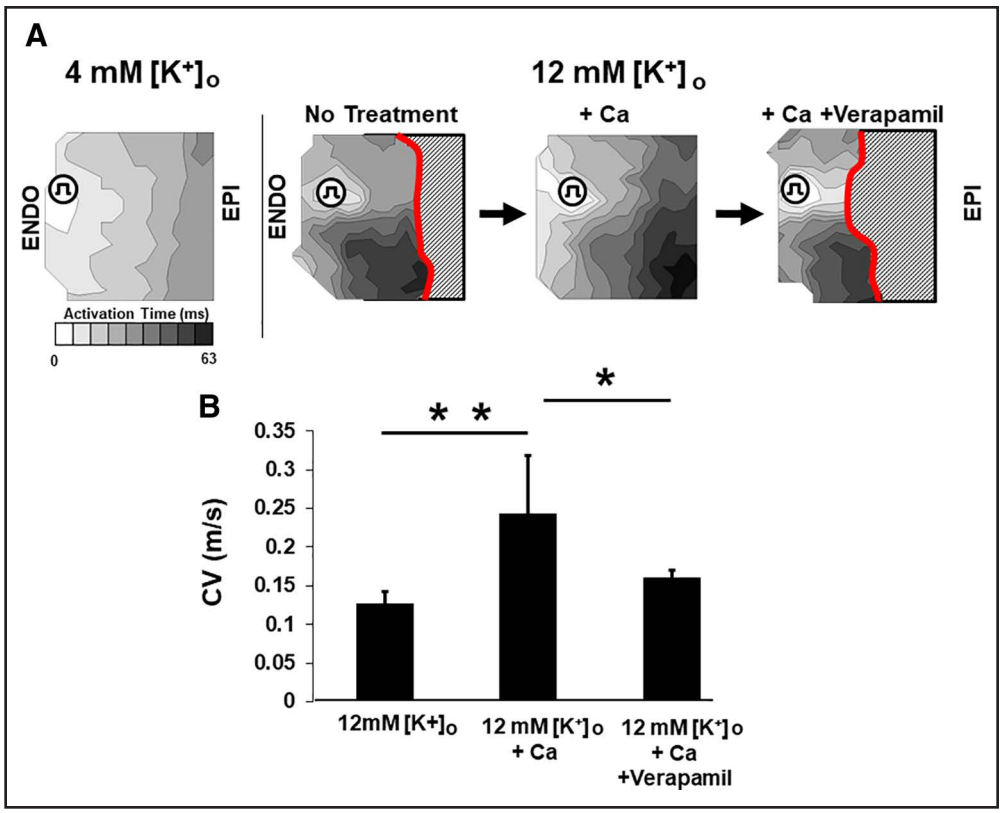

Figure 6. Effect of verapamil and calcium (Ca^{2+}) on transmural conduction. **A**, **Upper row** shows (in order *left* to *right)* transmural conduction maps at baseline, hyperkalemia, hyperkalemia + Ca^{2+} treatment, and hyperkalemia + Ca^{2+} treatment in the presence of L-type Ca^{2+} channel blockade with verapamil. Conduction slows during hyperkalemia and subsequent subepicardial conduction block is observed. Ca^{2+} treatment improves conduction and ameliorates conduction block. The addition of verapamil again slows conduction and conduction block returns. **B**, Summary data for conduction velocity (CV) during hyperkalemia and Ca^{2+} treatment, with and without verapamil, is shown (*$p = 0.02$, **$p < 0.001$, $n = 3$). ENDO = endocardial, EPI = epicardial.

T waves of the ECG in moderate elevations of potassium (1, 3). However, we did not observe that hyperkalemia promoted APD heterogeneities that would potentially promote reentrant arrhythmias, nor did Ca^{2+} treatment affect APD heterogeneities. This suggests that Ca^{2+} treatment would likely have minimal effect on RGs, other than those produced by conduction slowing.

We observed differences in the effect of Ca^{2+} treatment on APD in isolated myocytes vs. the wedge preparation and less sensitivity to hyperkalemia in the wedge preparation. Effects observed in isolated cells are known to be different from what is observed in multicellular preparations due to, in part, the effects of cell-to-cell coupling by gap junctions, which decreases intrinsic electrophysiologic heterogeneities in tissue. Therefore, known heterogeneities in myocyte sensitivity to hyperkalemia (5) may explain why the effects of hyperkalemia and Ca^{2+} treatment were more apparent in single-cell preparations. Importantly, our examination of effects in both isolated myocytes and intact tissue, therefore, provides additional insight into mechanisms relevant to the whole heart.

Limitations

To achieve ECG changes consistent with severe hyperkalemia, including a sine wave pattern ECG, we used 12 mM $[K^+]o$ to significantly slow CV, which may be less clinically relevant than the 6 mM $[K^+]o$ used in cellular studies. However, differences in susceptibility to hyperkalemia in experimental mammalian preparations and man have been noted before (1, 5). In addition, a biphasic response to hyperkalemia on cardiac conduction in multicellular preparations is well described, where significant conduction slowing is only observed at concentrations greater than 8 mM $[K^+]o$ in guinea pig and human (30–32). There are several limitations of the wedge preparation. Whole-heart Purkinje and bundle branch conduction are not taken into account, and potassium derangements have known preferential effects on the conduction system and Purkinje fibers (1, 4, 33), particularly at the Purkinje-myocyte junction (34). Nevertheless, the wedge preparation is an established model used to examine the electrophysiological heterogeneities and basis of the T wave under normal and pathologic conditions (13, 20, 35). Additionally, using the wedge preparation, we cannot comment on the effects of hyperkalemia on the SA or AV node and associated abnormal automaticity and bradycardia that occurs with hyperkalemia (1, 4, 7). Finally, we could not reliably induce arrhythmias or observe spontaneous arrhythmias in the wedge

preparation because of known size limitations and because of the inability to capture the preparation at short cycle lengths necessary to perform programmed electrical stimulation. A lack of specificity of tetrodotoxin for sodium currents and potential effect on L-type Ca^{2+} current has been suggested, but this is controversial (32, 36–39). We did not perform studies with verapamil or tetrodotoxin in the absence of hyperkalemia or in dose-response studies with Ca^{2+} treatment. We also did not test Ca^{2+} treatment at 8 mM $[K^+]o$.

Clinical Implications

Although these data provide a mechanistic rationale for Ca^{2+} treatment when there is evidence of hyperkalemia-induced conduction slowing, it should be noted that additional considerations are important when considering Ca^{2+} treatment. For example, there are well described side effects of Ca^{2+} administration, including skin injury if infiltrated and unpleasant hot flushes or chalky taste, but severe complications, such as hypotension, bradycardia, and arrhythmias are uncommon (10, 40). An additional consideration is that ECG changes in these patients may evolve quickly and patients may develop life-threatening complications, and the typical treatment error is failure to treat with Ca^{2+}, rather than overtreatment. However, there are data to suggest that in patients with adverse events, ECG changes are oftentimes present, and that these adverse events are not observed if only isolated T wave changes are present (41). Although it has been reported that in patients with hyperkalemia, there is often concomitant acidosis, this is not accounted for in these experiments. However the interaction between hyperkalemia, acidosis, and Ca^{2+} on conduction is complex, where acidosis slows conduction during hyperkalemia (31) but also increases ionized Ca^{2+} (42), which may mitigate effects of hyperkalemia on conduction.

CONCLUSIONS

These data provide evidence that Ca^{2+} treatment for hyperkalemia improves cardiac conduction through Ca^{2+}-mediated conduction, rather than improvement in RMP or "membrane stabilization." These data support the clinical practice of treating hyperkalemia with Ca^{2+} when the ECG reveals conduction slowing and QRS widening. It should be noted that due to our model, these data cannot inform its use during bradycardia, or other arrhythmias, as is currently clinically indicated.

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