Heterogeneous ventricular chamber response to hypokalemia and inward rectifier potassium channel blockade underlies bifurcated T wave in guinea pig

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Poelzing S, Veeraraghavan R. Heterogeneous ventricular chamber response to hypokalemia and inward rectifier potassium channel blockade underlies bifurcated T wave in guinea pig. Am J Physiol Heart Circ Physiol 292: H3043–H3051, 2007. First published February 16, 2007; doi:10.1152/ajpheart.01312.2006.—It was previously demonstrated that transmural electrophysiological heterogeneities can inscribe the ECG T wave. However, the bifurcated T wave caused by loss of inward rectifier potassium current (I\textsubscript{K1}) function is not fully explained by transmural heterogeneities. Since right ventricular (RV) guinea pig myocytes have significantly lower I\textsubscript{K1} than left ventricular (LV) myocytes, we hypothesized that the complex ECG can be inscribed by heterogeneous chamber-specific responses to hypokalemia and partial I\textsubscript{K1} blockade. Ratiometric optical action potentials were recorded from the epicardial surface of the RV and LV. BaCl\textsubscript{2} (10 μmol/l) was perfused to partially block I\textsubscript{K1} in isolated guinea pig whole heart preparations. BaCl\textsubscript{2} or hypokalemia alone significantly increased RV basal (RV\textsubscript{B}) action potential duration (APD) by ∼30% above control compared with LV apical (LV\textsubscript{A}) APD (14%, P < 0.05). In the presence of BaCl\textsubscript{2}, 2 mmol/l extracellular potassium (hypokalemia) further increased RV\textsubscript{B} APD to a greater extent (31%) than LV\textsubscript{A} APD (19%, P < 0.05) compared with BaCl\textsubscript{2} perfusion alone. Maximal dispersion between RV\textsubscript{B} and LV\textsubscript{A} APD increased by 105% (P < 0.05), and the QT interval prolonged by 55% (P < 0.05) during hypokalemia and BaCl\textsubscript{2}. Hypokalemia and BaCl\textsubscript{2} produced an ECG with a double repolarization wave. The first wave (QT1) corresponded to selective depression of apical LV plateau potentials, while the second wave (QT2) corresponded to the latest repolarizing RV\textsubscript{B} potentials. These data suggest that final repolarization is more sensitive to extracellular potassium changes in regions with reduced I\textsubscript{K1} particularly when I\textsubscript{K1} availability is reduced. Furthermore, underlying I\textsubscript{K1} heterogeneities can potentially contribute to the complex ECG during I\textsubscript{K1} loss of function and hypokalemia.

IT IS WELL ESTABLISHED THAT electrophysiological heterogeneities inscribe the distinct ECG morphology. The T wave, representing final repolarization, has been linked to dispersion of repolarization across the ventricular wall (40). However, the preferential appearance of bifurcated T waves in leads II and V\textsubscript{3} during hypokalemia (39) suggests that the predominant heterogeneity during hypokalemia may be regional rather than transmural.

Interestingly, hypokalemia has dichotomous effects on repolarizing potassium currents. For example, the inward rectifier potassium current (I\textsubscript{K1}) has a paradoxical relationship with extracellular K\textsuperscript{+} concentration ([K\textsuperscript{+}]\textsubscript{o}) where I\textsubscript{K1} peak current density is reduced during hypokalemia (28). I\textsubscript{K1} plays an important role in ventricular action potentials by modulating resting membrane potential and final repolarization. In humans, I\textsubscript{K1} reduction, as occurs in Andersen–Tawil syndrome type I (ATS1), is associated with prominent secondary repolarization ECG waves (U waves) particularly during hypokalemia (41, 42). While the functional I\textsubscript{K1} protein distribution in humans remains unknown, I\textsubscript{K1} heterogeneities have been demonstrated previously in a variety of other animal models (8, 37). However, it is unclear what role I\textsubscript{K1} heterogeneities have in the inscription of the ECG, particularly under conditions in which I\textsubscript{K1} is partially blocked or altered by lowering extracellular potassium.

Previous studies of partial I\textsubscript{K1} blockade have been unable to recapitulate a bifurcated T wave with or without hypokalemia (29, 33). Interestingly, these models of partial I\textsubscript{K1} blockade only explored the contribution of left ventricular (LV) transmural heterogeneities to the ECG’s inscription. Since right ventricular (RV) guinea pig myocytes have significantly lower I\textsubscript{K1} than LV myocytes (37), we hypothesized that the complex ECG can be inscribed by heterogeneous chamber-specific responses to hypokalemia and partial I\textsubscript{K1} blockade.

In this study we demonstrate that the response to hypokalemia and partial I\textsubscript{K1} blockade is heterogeneous between the RV and LV in guinea pig. In general, action potential duration (APD) of myocytes with reduced I\textsubscript{K1} (RV) prolongs significantly more than those with more I\textsubscript{K1} (LV) during hypokalemia and partial I\textsubscript{K1} blockade. This ventricular chamber-specific heterogeneity manifests as a complex ECG repolarization morphology arising from differences in action potential trajectories. Specifically, the first wave after depolarization, labeled T1, is associated with prominent secondary repolarization ECG waves (U waves) particularly during hypokalemia (41, 42). While the functional I\textsubscript{K1} protein distribution in humans remains unknown, I\textsubscript{K1} heterogeneities have been demonstrated previously in a variety of other animal models (8, 37). However, it is unclear what role I\textsubscript{K1} heterogeneities have in the inscription of the ECG, particularly under conditions in which I\textsubscript{K1} is partially blocked or altered by lowering extracellular potassium.

METHODS

Experimental preparation. The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Pub. No. 85-23, Revised 1996) and the Guide to the Care and Use of Experimental Animals.
has been approved by the Institutional Animal Care and Use Committee of the University of Utah (protocol no. 05-07002). Retired breeder male guinea pigs were anesthetized (30 mg/kg pentobarbital sodium (Nembutal) ip; n = 19), and their hearts were rapidly excised and perfused as Langendorff preparations (perfusion pressure 55 mmHg) with oxygenated (100% O₂) Tyrode solution at 36.5°C containing (mmol/l) 2 CaCl₂, 140 NaCl, 4.5 KCl, 10 dextrose, 1 MgCl₂, and 10 HEPES (pH 7.40). Preparations were completely immersed in temperature-controlled (36 ± 1°C) perfusate to prevent the formation of regional temperature gradients. The right and left atria were excised to avoid competitive stimulation. Hearts were stained with the voltage-sensitive dye di-4-ANEPPS (15 μmol/l) by direct coronary perfusion for 10 min.

Ratiometric optical mapping system. To reduce motion artifacts and more accurately reconstruct transmembrane potentials, we developed a ratiometric optical action potential mapping system similar to that of Himel and Knisley (13). Specifically, we used two SciMedia MiCam02 HS charge-coupled device (CCD) cameras (SciMedia, Irvine, CA) in a tandem lens configuration capable of resolving membrane potential changes with 1-ms temporal resolution from 90 × 60 sites simultaneously sampled with a 14-bit analog-to-digital converter. After staining with the voltage-sensitive dye di-4-ANEPPS, the preparation was excited by two 60-LED light sources (RL5-A9018, Superbrightteds, St. Louis, MO) powered by a custom-built 15-V DC power supply (3 0 mA per LED). Each light source was fitted with an equal optical path lengths. The interpixel resolution was 0.184 mm in the y-direction (60 pixels) and 0.199 mm in the x-direction (90 pixels). The relative change in voltage (V_r) was defined as V_r = ΔF/S0/ΔF_S0, where ΔF represents the change in fluorescence for a particular wavelength.

Optical action potential measurements. Motion was further reduced by perfusion of 7.5 mmol/l 2,3-butanedione monoxime. Hearts were stimulated with a unipolar silver wire placed on the basal epicardial septum at 1.5 times the stimulation threshold with basic cycle lengths (BCL) of 500 Hz down to loss of 1:1 capture. Activation time was defined as the time of the maximum first derivative of the action potential as described previously (11). Repolarization was defined as the time at which the amplitude of the voltage transient reached 95% repolarization from peak voltage amplitude. APD was the time difference between activation and repolarization.

All recordings were divided into eight regions from the LV and RV corresponding to their anatomic location (anterior: RV base, RV apex, LV base, LV apex; posterior: RV base, RV apex, LV base, LV apex). Each region consisted of a minimum of 400 optical sites. Regional APD was quantified as the average regional APD. Anterior and posterior measurements were made sequentially by turning the heart in the imaging chamber in order to record from appropriate sites.

ECG measurements. The bath ECG was obtained with a silver chloride anode located ~2 cm from the midwall of the RV and a cathode located ~2 cm from the midwall of the LV. QT1 and QT2 were calculated as the time difference between initial depolarization following the stimulus and the end of the T1 or T2 wave. The QT1 and subsequent QT2 intervals were plotted, and regression analysis was performed for each QT correction equation obtained from Malik et al. (22). The hyperbolic QT correction formula [QTc = QT + (1/BCL - 1)] demonstrated the best fit for all measurements (slope < 0.1, P < 0.01) and was therefore used to compare the QT/QU relationship over all BCL.

Pharmacological intervention. Since BaCl₂ is a relatively specific IK₁ blocker at micromolar doses (30), we varied extracellular BaCl₂ from 10 to 100 μmol/l (n = 3). A final concentration of 10 μmol/l BaCl₂ (n = 6) was chosen to determine the role of [K⁺]o on action potential trajectories during IK₁ blockade. This concentration is similar to that used in a canine model of IK₁ blockade (33) and a guinea pig model of ventricular fibrillation (37). We varied [K⁺]o in the presence and absence of BaCl₂ (n = 6 additional experiments). All optical recordings were made after 15 min of BaCl₂ and/or 2 (hypokalemic), 4.5 (normokalemic), or 6 (hyperkalemic) mmol/l [K⁺]o perfusion. All interventions were compared with control conditions (n = 4).

Arrhythmia induction. To assess changes in susceptibility to arrhythmias during all experimental conditions, programmed electrical stimulation was performed according to an identical protocol on all preparations. After a 20-beat drive train delivered to the anterior epicardial surface of either the RV or LV (BCL of 400 ms), an epicardial premature stimulus (S2) was delivered through the same drive train electrode at a S1-S2 coupling interval of 300 ms. The S1-S2 interval was sequentially shortened by 10-ms decrements until refractoriness was reached or an arrhythmia was induced. Subsequently, a 20-beat drive train was delivered at a BCL 20 ms longer than the shortest pacing rate capable of 1:1 capture in order to observe arrhythmia susceptibility to rapid pacing.

Statistical analysis. Statistical analysis of the data was performed with a two-tailed Student’s t-test for paired and unpaired data or a single-factor ANOVA. A P < 0.05 was considered statistically significant. All values are reported as means ± SE unless otherwise noted.

RESULTS

Regional APD heterogeneity. For all control experiments anterior RV basal (RVB) APD was significantly greater than that in all other regions of the heart (Table 1). Importantly, anterior RVB and anterior RV apical APD were significantly greater than the corresponding posterior regions (posterior RV base and posterior RV apex). In the LV, only the anterior LV

| Table 1. Regional APD under control conditions and during perfusion of 10 μmol/l BaCl₂ |
|-----------------------------------|-------------------|
| Control                           | 163 ± 8†          |
| Anterior right base               | 151 ± 7           |
| Anterior right apex               | 158 ± 7           |
| Anterior left base                | 142 ± 7†          |
| Anterior left apex                | 154 ± 6           |
| Posterior right base              | 148 ± 7          |
| Posterior right apex              | 153 ± 8†          |
| Posterior left base               | 142 ± 8           |
| Posterior left apex               | 189 ± 5†          |
| 10 μmol/l BaCl₂                   | 188 ± 5           |
| Anterior right base               | 182 ± 11          |
| Anterior right apex               | 168 ± 7           |
| Anterior left base                | 177 ± 7†          |
| Anterior left apex                | 177 ± 7           |
| Posterior right apex              | 172 ± 5†          |
| Posterior left base               | 169 ± 6           |

Values are means ± SE. APD, action potential duration. *P < 0.05 compared with corresponding anterior region; †P < 0.05 compared with all other regions.
basal (LV_B) APD was significantly greater than the posterior LV_B APD. Extracellular BaCl_2 was varied from 10 to 100 μmol/l in order to determine the regional heterogeneity of APD during I_{K1} blockade. Representative action potentials in Fig. 1A demonstrate the effect of 30 and 100 μmol/l BaCl_2. Specifically, APD for all cell types significantly prolonged with increased BaCl_2 (BCL = 350 ms). Furthermore, the QT interval obtained from the bath ECG prolonged with increased BaCl_2 as demonstrated in Fig. 1A. The graph in Fig. 1B demonstrates that the mean APD for all experiments increases as the concentration of BaCl_2 is increased. The APD maximum regional dispersion likewise increased as BaCl_2 concentration increased (Fig. 1C).

To compare these results with previous canine models of partial I_{K1} blockade (33) and electrophysiological changes associated with BaCl_2 perfusion in guinea pig ventricles (37), a 10 μmol/l dose of BaCl_2 was used for subsequent experiments. The regional distribution pattern of APD remained similar during BaCl_2 perfusion (Table 1). However, APD significantly prolonged for all regions (P < 0.05). Additionally, maximal APD dispersion occurred between the anterior RV_B and the anterior LV apex (LV_A) (Table 1) and was increased compared with control by 54%.

**Hypokalemia and I_{K1} blockade increase interventricular heterogeneity.** Since extracellular potassium fluctuations are often associated with ventricular ectopy, extracellular potassium was varied to 6 and 2 mmol/l [K^+]_o in the presence of 10 μmol/l BaCl_2. Representative action potentials in Fig. 2A demonstrate the change in APD from corresponding sites in Fig. 1A. For all regional cell types, BaCl_2 (Fig. 2A, red traces) increased APD above control (Fig. 2A, black traces). During BaCl_2 perfusion, APD varied inversely with [K^+]_o. Specifically, 6 mmol/l [K^+]_o + BaCl_2 (Fig. 2A, blue traces) decreased APD for all cell types compared with 4.5 mmol/l [K^+]_o + BaCl_2. On the other hand, 2 mmol/l [K^+]_o + BaCl_2 (Fig. 2A, green traces) increased APD for all regions compared with control and 4.5 mmol/l [K^+]_o + BaCl_2.

The representative APD maps in Fig. 2B demonstrate the spatial distribution of APD on the anterior surface of the heart during experimental conditions. Maps were scaled such that the APD range for all experimental conditions in Fig. 2B is constant (45 ms). The anterior RV_B manifests the longest APD.

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**Fig. 1.** Inward rectifier potassium current (I_{K1}) blockade increases action potential duration (APD) and APD dispersion. A: representative action potentials from the anterior surface of guinea pig ventricle. Increased doses of BaCl_2 increase APD for all epicardial cell types. Increased APD is associated with increased ECG QT interval. B: mean APD for the entire anterior epicardial surface increases with increased concentration of BaCl_2. C: regional APD dispersion [left ventricular apex (LV_A) subtracted from right ventricular base (RV_B) APD] increases with blockade of I_{K1}. *P < 0.05 compared with 0 μmol/l BaCl_2.
compared with all other regions of the heart, as mentioned above. Additionally, BaCl₂ significantly increased APD for all regions (Fig. 2B, top right) as demonstrated by an increase in sites with the shortest APD from 150 ms under control conditions to 170 ms. BaCl₂ increased APD dispersion from the control value of 23 ms to 35 ms. Hyperkalemia (6 mmol/l \([\text{K}^+]_o\)) and BaCl₂ decreased regional dispersion back to 22 ms (Fig. 2B, bottom right) while maintaining a significantly greater average APD over control. Importantly, 2 mmol/l \([\text{K}^+]_o\) + BaCl₂ further increased APD for all cell types, consistent with the paradoxical dependence of \(I_{K1}\) (12) and the rapid component of delayed rectifier potassium current (\(I_{Kr}\)) (27) on \([\text{K}^+]_o\). Additionally, APD dispersion increased to 45 ms (Fig. 2B, bottom left).

Maximum APD dispersion occurred between anterior RVₐ and anterior LVₐ for all experiments and conditions. In the results described below, RVₐ refers to all action potentials recorded from the anterior RVₐ, and LVₐ refers to all action potentials recorded from the anterior LVₐ.

Summary data in Fig. 3A demonstrate that under baseline conditions RVₐ APD is significantly greater than LVₐ APD for all \([\text{K}^+]_o\) (\(P < 0.05\)). BaCl₂ significantly increased APD in both regions for all \([\text{K}^+]_o\) (\(P < 0.05\)). However, RVₐ APD was significantly greater than LVₐ APD only during perfusion of 2 and 4.5 mmol/l \([\text{K}^+]_o\). Hyperkalemia attenuated regional APD heterogeneities in the presence of BaCl₂. Regional APD dispersion is summarized in Fig. 3B. Hypokalemia in the absence of BaCl₂ significantly increased APD dispersion above 4.5 mmol/l \([\text{K}^+]_o\) by \(\sim 50\%\). However, hyperkalemia alone has no observable affect on APD dispersion. BaCl₂ increased APD dispersion under conditions of 2 and 4.5 mmol/l \([\text{K}^+]_o\). Specifically, hypokalemia increased APD dispersion by \(\sim 105\%\) during BaCl₂ perfusion, while 4.5 mmol/l \([\text{K}^+]_o\) + BaCl₂ increased APD to a similar extent as hypoka-
significantly change APD dispersion in the presence of 10 mmol/l BaCl₂. APD for all cell types demonstrates an inverse relationship to \([K^+]_o\). This inverse dependence is increased during perfusion of BaCl₂. RV_B APD is greater than LV_A APD in control and BaCl₂-perfused experiments\((\ddagger P < 0.05)\) except during 6 mmol/l \([K^+]_o\) + BaCl₂. BaCl₂ increases all APD above baseline for all cell types\((* P < 0.05)\) except during 6 mmol/l \([K^+]_o\) alone and 2 mmol/l \([K^+]_o\) + BaCl₂. This relationship is also present during perfusion of BaCl₂. Addition of BaCl₂ significantly increases all regional APD differences\((* P < 0.01)\) for 2 and 4.5 mmol/l \([K^+]_o\).

ECG manifestations during hypokalemia and \(I_{Kr}\) blockade. The measurement of the QT interval obtained from the bath ECG during control conditions is illustrated in Fig. 4A. Under control conditions, pacing from the anterior basal septum produces a bath ECG pattern with a well-defined QRS and T-wave morphology (Fig. 4A). Hypokalemia (Fig. 4B) prolongs the QT interval and causes ST-segment depression. The addition of BaCl₂ under normokalemic conditions increases the QT interval but otherwise does not significantly change the morphology of the bath ECG (Fig. 4C). Hypokalemia and BaCl₂ are associated with ST-segment depression and the appearance of an additional repolarizing wave labeled T2 in Fig. 4D. However, the end of T2 in this example is obscured by the pacing artifact for the subsequent beat. Prolonging the BCL to 400 ms (Fig. 4E) and 500 ms (Fig. 4F) ms reveals final repolarization of T2.

QT1c for all experiments and pacing rates from 500- to 300-ms BCL are summarized in Fig. 5A. Normokalemia with BaCl₂ significantly increased QT1c from 185 ± 4 ms under control conditions to 225 ± 2 ms\((P < 0.001)\). Hypokalemia with BaCl₂ did not significantly change QT1c \((184 ± 5 \text{ ms})\) compared with control. The QT2c interval under conditions of hypokalemia with BaCl₂ significantly increased above control and normokalemia with BaCl₂ perfusion. The latest repolarization times (top 95th percentile) in the RV_B \((285 ± 13 \text{ ms})\) closely corresponded to QT2c \((280 ± 12 \text{ ms}, \text{Fig. 5B})\). However, the earliest repolarization times (lowest 5th percentile) in the LV_A \((251 ± 13 \text{ ms})\) were significantly longer than QT1c\((P < 0.05)\).

Representative action potentials from RV_B and LV_A during 2 mmol/l \([K^+]_o\) + BaCl₂ perfusion are superimposed in Fig. 5C. The first phase of repolarization in LV_A sites is significantly faster than that in RV_B sites. Digital subtraction of these two traces reveals a double repolarization wave difference. This double wave corresponds well with the double repolarization wave observed in the bath ECG under these conditions. Therefore, these data suggest that T1, under conditions of 2 mmol/l \([K^+]_o \) + BaCl₂ perfusion, represents interventricular plateau heterogeneities and T2 represents final repolarization.

Action potential trajectory changes independent of \(I_{Kr}\). Varying extracellular potassium has dichotomous effects on repolarizing currents. Specifically, \(I_{Kr}\) has a paradoxical relationship with \([K^+]_o\), in which low \([K^+]_o\) decreases \(I_{Kr}\). On the other hand, the slow component of the delayed rectifier potassium current \((I_{Kr})\) has an ohmic relationship with \([K^+]_o\), in which low \([K^+]_o\) increases \(I_{Kr}\). Since \(I_{Kr}\) has a paradoxical relationship to \([K^+]_o\), our results with hypokalemia and BaCl₂ may also unveil heterogeneities due to partial \(I_{Kr}\) block and produce an complex ECG. Therefore, it was important to determine the effects of \(I_{Kr}\) blockade during hypokalemia on the ECG.

Regional APD differences during hypokalemia, represented as APD dispersion in Fig. 6A, significantly increased with the addition of BaCl₂. The underlying mechanism of increased APD dispersion is a result of hypokalemia and BaCl₂ significantly prolonging RV_B APD to a greater extent than LV_A APD (Fig. 3A; \(P < 0.05\)). Terfenadine, a potent \(I_{Kr}\) blocker, and 2 mmol/l \([K^+]_o\), also significantly increased regional APD dispersion above 2 mmol/l \([K^+]_o\) alone and 2 mmol/l \([K^+]_o\) + BaCl₂ (Fig. 6B). The largest dispersion in this experiment likewise occurred between RV_B and LV_A. Representative action potentials from RV_B and LV_A are superimposed in Fig. 6B. The trajectories of the two action potentials are similar. Action potentials in both regions appear more triangulated, and terfenadine and hypokalemia increase RV_B APD to a greater extent than LV_A APD. The action potential difference
trace and bath ECG (Fig. 6B) demonstrate a single repolarization wave.

**Ventricular arrhythmias.** Arrhythmias were neither spontaneous nor initiated with programmed electrical stimulation during perfusion of BaCl2 or hypokalemia alone. However, during hypokalemia and BaCl2, spontaneous arrhythmias occurred in three of six preparations. Representative bath ECGs in Fig. 4, G and H, demonstrate a spontaneous polymorphic arrhythmia and a rapid pacing-induced monomorphic arrhythmia, respectively. The last stimulus (S1, BCL = 150 ms) present in Fig. 4H. All arrhythmias were self-terminating. Arrhythmias were never induced with programmed stimulation (up to 2 extra premature beats) during hypokalemia and BaCl2. Relatively rapid pacing (between 310- and 250-ms BCL) produced from three extra beats to 30 s of spontaneously terminating monomorphic ventricular tachycardia in five of six preparations with hypokalemia and BaCl2. In contrast, rapid pacing did not produce any tightly coupled spontaneous beats under control conditions.

**DISCUSSION**

The purpose of this study was to test the hypothesis that the complex ECG can be inscribed by heterogeneous responses to hypokalemia and partial I_{K1} blockade due to underlying I_{K1} heterogeneities. We observed the greatest differences in APD between the anterior RVB and the anterior LV A under all experimental conditions, consistent with reduced RV I_{K1} expression (26). These data of regional APD heterogeneities from the anterior epicardial surface of guinea pig ventricles under control conditions are consistent with previous reports (19, 38). Hypokalemia, BaCl2, or terfenadine alone increased RVB APD, which corresponded well with QT prolongation. Despite QT prolongation, these interventions did not alter the ECG morphology. However, combining hypokalemia with partial I_{K1} blockade produced a complex ECG with two repolarization waves. Specifically, the first repolarization wave (QT1c) was not significantly different from control, while the second ECG repolarization wave (QT2c) became prominent and was significantly longer than QTc and QT1c for all experimental conditions. In this model of hypokalemia and partial I_{K1} blockade, QT1 corresponded to selective depression of apical LV plateau potentials compared with other regions, while QT2 corresponded to disproportionate prolongation of RVB repolarization times compared with LV A. These data suggest that final repolarization of myocytes in regions with reduced I_{K1} expression (RVB myocytes in the case of the guinea pig) may be more sensitive to changes in [K+]o under conditions of reduced I_{K1} availability. Furthermore, the complex ECG repolarization waves may be a result of selective difference in action potential trajectory and not just final repolarization under conditions of hypokalemia and reduced I_{K1}.

It was demonstrated previously that heterogeneous transmural action potential trajectory differences in the canine wedge preparation can inscribe a bifurcated T wave during sotalol perfusion with (40) or without (41) hypokalemia. While the electrophysiological heterogeneity was transmural rather than...
between chambers, the mechanism of action potential trajectory differences is consistent with the mechanism of a bifurcated T wave observed in this study. However, a bifurcated T wave was not observed in the canine wedge preparation under conditions of hypokalemia and partial $I_{K1}$ blockade (33).

It is important to note that the complex T wave may have more than one underlying mechanism depending on the experimental model or disease. For example, the second component of a bifurcated T wave has been referred to as a U wave in the literature (40). U waves have been observed in patients with hypokalemia (39), catecholaminergic polymorphic ventricular tachycardia (1), ischemia (7, 10, 23), and ventricular hypertrophy and dilatation (24, 34), to name a few conditions. Additionally, U waves manifest in normal subjects (21), further adding to the controversial nature of this wave. It has been postulated that muscle movement and stretch-activated channels may contribute to delayed afterdepolarizations and subsequently the U wave (32, 35). Muscular contraction and the distribution of stretch-activated channels may also be regionally heterogeneous. Therefore, the underlying mechanism of the U wave could be a result of delayed afterdepolarizations or heterogeneous repolarization. This study is consistent with previous reports that suggest the second component of the bifurcated T wave in guinea pig during hypokalemia and $I_{K1}$ blockade is a “resumption of an interrupted T wave” (40), warranting the label “bifurcated T wave” instead of “U wave.” Therefore, a broader investigation of the entire ventricular myocardium may be necessary to determine the underlying mechanisms of bifurcated T waves compared with U waves.

$I_{K1}$ and heterogeneous chamber-specific responses. The heterogeneous ventricular chamber-specific response to hypokalemia and partial $I_{K1}$ blockade is likely related to the reduced RV inward rectifier potassium channel ($K_{ir}$)2.1 and Kir2.3 expression compared with LV previously demonstrated in guinea pig (26, 37). However, $I_{K1}$ distribution seems to be
species dependent. Specifically, there are no measurable \( I_{K1} \) differences in canine myocardium either transmurally (20) or between ventricles (36), while \( I_{K1} \) is heterogeneously distributed transmurally in feline myocardium (8). Therefore, blocking \( I_{K1} \) may have heterogeneous effects regionally or transmurally depending on the animal model chosen. In general, these data suggest that \( I_{K1} \) heterogeneities may play a larger role in electrophysiological heterogeneities and the inscription of the ECG than previously proposed.

**Arrhythmia mechanisms.** While total APD dispersion was significantly increased under conditions of hypokalemia, BaCl\(_2\), terfenadine, or the combination of hypokalemia and BaCl\(_2\), the magnitude of dispersion did not exceed 50 ms. Specifically, gradients of repolarization between the RV and LV were <5 ms/mm, significantly lower than the proposed value of 10 ms/mm that was previously found to be critical for the development of conduction block and reentry (2). These data are consistent with our inability to initiate arrhythmias with programmed stimulation. Furthermore, the observed interventricular and apicobasal heterogeneities may have been an insufficient substrate for arrhythmias in guinea pig, and therefore it may be that these gradients are even less sufficient to serve as a substrate for arrhythmias in the case of larger hearts since the repolarization gradients between ventricles may be less steep. A previous study of \( I_{K1} \) blockade in the canine wedge preparation demonstrated that LV transmural gradients of repolarization were also insufficient to induce arrhythmias, but triggered activity was increased during hypokalemia and partial \( I_{K1} \) blockade (33).

Hypokalemia and cycle length prolongation before arrhythmia initiation, on the other hand, are common clinical features of drug-induced tordos de points (5, 25, 31), where afterdepolarization activity increases and serves as the arrhythmia trigger. We observed spontaneous arrhythmias only during hypokalemia and partial \( I_{K1} \) blockade. However, it remains unknown whether the spontaneous arrhythmias were reentrant or focal in nature. In this study, the initiations of monomorphic arrhythmias via rapid pacing only occurred during hypokalemia and partial \( I_{K1} \) blockade, which is in conflict with bradycardia-induced tordos de points. Rapid pacing is also associated with triggered activity due to elevation of diastolic calcium levels (18), and hypokalemia alone can raise intracellular diastolic calcium levels and thereby increase triggered activity (9). While \( I_{K1} \) downregulation up to ~80% is sufficient to significantly increase pacemaker activity in ventricular myocytes, it remains unknown whether a much smaller degree of \( I_{K1} \) block can lower the threshold for triggered activity, particularly during hypokalemia when \( I_{K1} \) is further reduced. This study suggests that arrhythmias during hypokalemia and partial \( I_{K1} \) blockade may be a result of triggered activity and less likely dependent on large gradients of repolarization acting as a substrate for reentrant arrhythmias.

**Limitations.** While BaCl\(_2\) is a relatively potent \( I_{K1} \) blocker, barium alters calcium-mediated inactivation of the L-type calcium channel (6). However, this study used doses of BaCl\(_2\) orders of magnitude smaller (\( \mu \)mol/l) than those reported to significantly alter L-type calcium channel kinetics (mmol/l) (3). Like all nonspecific blockers, the potential for nonspecific electrophysiological effects may complicate interpretation. Therefore, this study should not be considered a direct model of any specific \( I_{K1} \) loss-of-function disease. Instead, these results highlight the importance of considering chamber-specific heterogeneities that can underlie complex ECG phenom-on.

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