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Pro-arrhythmic Effects of Low Plasma [K^+] in Human Ventricle: An Illustrated Review

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Short Title: Low $[K^+]_o$ and Human Ventricular Arrhythmias

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Abstract

Potassium levels in the plasma, $[K^+]_o$, are regulated precisely under physiological conditions. However, increases (from approx. 4.5 to 8.0 mM) can occur as a consequence of e.g. endurance exercise, ischemic insult or kidney failure. This hyperkalemic modulation of ventricular electrophysiology has been studied extensively. Hypokalemia is also common. It can occur in response to diuretic therapy, following renal dialysis, or during recovery from endurance exercise. In the human ventricle, clinical hypokalemia (e.g. $[K^+]_o$ levels of approx. 3.0 mM) can cause marked changes in both the resting potential and the action potential waveform, and these may promote arrhythmias. Here, we provide essential background information concerning the main K^+-sensitive ion channel mechanisms that act in concert to produce prominent short-term ventricular electrophysiological changes, and illustrate these by implementing recent mathematical models of the human ventricular action potential.

Even small changes (~1mM) in $[K^+]_o$ result in significant alterations in two different K^+ currents, I_{K1} and HERG. These changes can markedly alter in resting membrane potential and/or action potential waveform in human ventricle. Specifically, a reduction in net outward transmembrane K^+ currents (repolarization reserve) and an increased substrate input resistance contribute to electrophysiological instability during the plateau of the action potential and may promote pro-arrhythmic early after-depolarizations (EADs). Translational settings where these insights apply include: optimal diuretic therapy, and the interpretation of data from Phase II and III trials for anti-arrhythmic drug candidates.

Background:

Rapid and effective regulation of plasma K^+ levels, $[K^+]_o$, within a narrow range (3.5 - 5 mM) is a fundamental physiological principle in Cardiovascular Physiology.[1] In most mammalian tissues the electrochemical gradient for K^+ sets the resting potential. As a consequence, changes in $[K^+]_o$ modulate essential parameters such as excitability and action potential duration (APD), as well as intracellular Ca^{2+}, $[Ca^{2+}]_i$, and (thus) both excitation-contraction and excitation-secretion coupling. The effects of deviations from normal $[K^+]_o$ levels have been studied in detail in both physiological[2,3] and pathophysiological settings.[4,5] As examples, in the heart the relatively short term increase in $[K^+]_o$ that results from either maintained periods of high rates,[3] or a localized ischemic insult,[4] have been well characterized.

Importantly, however, $[K^+]_o$ can also decrease significantly for extended periods of time. This hypokalemia occurs in settings such as recovery from endurance exercise, or in the immediate post-dialysis time period in patients being treated for renal failure. Low $[K^+]_o$ has also been reported in a number of increasingly common clinical settings when patients receive diuretic therapy as part of heart failure or hypertension treatments.[6,7] Hypokalemia can occur even when this essential intervention is accompanied by K^+ replacement, (so-called 'Slow K^+' case management). It often results in muscle cramping and can also produce supraventricular rhythm disturbances.[8] In addition, $[K^+]_o$ regulation in the rapidly growing healthy aging population is an increasing clinical challenge partly because very commonly used

therapeutic agents can alter $[K^+]_o$ levels[8], and this sometimes changes cardiac electrophysiology in a patient-specific fashion.[9]

The main purpose of this illustrated review is to further explore the direct electrophysiological consequences of somewhat reduced, but still physiological $[K^+]_o$ levels of in the human ventricle. At the outset, it is necessary to determine what is meant by 'low physiological levels' of $[K^+]_o$. Recent reviews establish that normal plasma K^+ activities fall in the range 3.9 - 4.2 mM. $[K^+]_o$ levels below approximately 3.5 mM are defined as hypokalemic; and $[K^+]_o$ levels in excess of 6.0 mM are considered to be hyperkalemic[10,11]

Most of the physiological principles and molecular mechanisms by which $[K^+]_o$ can alter the electrophysiological activity in the mammalian heart (including the human) are quite well known.[12,13] However, some of these are not applied optimally in clinical settings or drug safety initiatives. The main K^+ selective ion channel that senses and reacts strongly to small changes in $[K^+]_o$ is the background inwardly rectifying K^+ current, I_{K1}.[12-15] This highly nonlinear current changes in an unexpected (or anomalous) way in response to changes in $[K^+]_o$.[16,17] Specifically, when $[K^+]_o$ is decreased (although the driving force for K^+ *increases* at the level of the resting potential), the outward current flow that is essential for maintaining the resting potential *decreases*. The immediate consequences are: (i) a small hyperpolarization, (ii) an altered AP waveform,[12, 14] (iii) a marked change in the input resistance at the resting potential,[17] and (iv) a change in the impedance profile (input resistance) during the plateau of the AP[18,19] This increase in impedance at the plateau of the AP alters (enhances) intercellular coupling in the atria, ventricles, and Purkinje conduction tissue. Previously, many of these principles were

incorporated into a mathematical model of the guinea pig ventricular action potential (Luo and Rudy,1991)[20] and their consequences on the resting potential, action potential waveform and excitability were explored in detail.

A second mechanism by which the intrinsic electrophysiological properties of the mammalian myocardium (mainly the ventricles) are changed in response to low $[K^+]_o$ is via its influences or the time- and voltage-sensitive K^+ current HERG.[21,22] Even small alterations in $[K^+]_o$ can be detected by these 'delayed rectifier K^+ channels'. Once again, although decreased $[K^+]_o$ increases electrochemical driving force for K^+, HERG channels generate a smaller outward current at the level of the plateau in low $[K^+]_o$[23]. The mechanism for this important but complex change has been identified; small changes in $[K^+]_o$ can alter the rapid, voltage-dependent inactivation of HERG channels.[24-27]

Most previous accounts of low $[K^+]_o$ effects have assumed that the myocardium consists of homogenous populations of myocytes and I_{K1} expression levels. However, it is now known that I_{K1} is expressed in both fibroblasts and myofibroblasts.[28] These cells make connexin-based contacts with other fibroblasts and perhaps also with myocytes.[28] Thus, electrotonic effects of low $[K^+]_o$ on action potential duration (APD) and excitability in both atria and ventricles, perhaps sensed/transduced by fibroblasts, are expected to contribute to the well-known pro-arrhythmic tendency of low $[K^+]_o$.

In this review, we illustrate and document these low $[K^+]_o$ induced electrophysiological changes by utilizing mathematical modeling. These simulations reveal an integrated pattern of results that can explain the unique ability of inwardly rectifying K^+ channels (I_{K1} and HERG) to detect even very small $[K^+]_o$ decreases and

rapidly change their properties (inactivation characteristics, and ion transfer relationships or current-voltage (I-V) curves, respectively). The net outward current in the physiological range of potentials is reduced in low $[K^+]_o$, and this small but functionally important decrease can alter both the resting potential and the repolarization waveform of the AP.[20,21] We emphasize these changes as an essential common (but perhaps not well appreciated) mechanism for short-term electrophysiological responses to hypokalemia. We conclude by drawing attention to the possibility that relatively frequent observations that may appear to be proarrhythmic drug or drug candidate actions, can be partly a result of off-target efforts of alterations in $[K^+]_o$ in atrial and ventricular substrates. [30-33]

Even Small Changes in $[K^+]_o$ have Significant Effects

Changes in $[K^+]_o$ as small as +/- 1.5 mM from the physiological level (approx.. 4.2 mM) can significantly alter the electrophysiological properties of human atria and ventricles. These effects are illustrated in Figure 1 in terms of the time-independent or background current (I_{K1}); the two main time-and voltage-dependent or delayed rectifier currents, HERG or I_{Kr} and I_{KS}; and the electrogenic current produced by the Na^+/K^+ pump in human ventricular myocytes. The four superimposed AP's in Panel A of Fig. 1 were obtained using our modified version of the classical ORd model of the human ventricular action potential developed by the Rudy Group.[33] This is described in detail in the Supplement Section.

Perhaps the most prominent changes in the K^+ currents that underlie the resting potential and action potential are those due to I_{K1}. This highly nonlinear background K^+

current changes almost instantly following changes in $[K^+]_o$ in the +/- 2mM range. Thus, a *decrease* in $[K^+]_o$ within the physiological range (e.g. 4.2 to 3.0 mM), results in an anomalous *decrease* in some functionally important K^+ currents (I_{K1} and HERG, Figs. 1 and S-1 and S-2) in human ventricle. In a variety of cells and tissue subtypes, decreasing $[K^+]_o$ also results in a marked change in the range of membrane voltage over which these K^+-selective channels are active. This so-called 'cross-over effect' of the ion transfer or current-voltage relationship is a hallmark feature of the strongly inwardly rectifying K^+ currents that are expressed in heart,[12,15, 20] in skeletal muscle[16] and also in smooth muscle myocytes from resistance vessels,[35,36] in both endothelial cells, and vascular pericytes.[37]

These changes are apparent both under control conditions (either 5.4 or 4.2 mM), and in response to the physiological increases in $[K^+]_o$ (8 mM), or decreases in plasma $[K^+]_o$ that have been identified in clinical settings and in individuals that are recovering from bouts of endurance exercise.[2,3]

[Fig. 1 near here]

In principle, a comprehensive understanding of the effects of alterations in plasma $[K^+]_o$ on the resting potential and AP waveform also requires consideration of another time- and voltage-dependent delayed rectifier K^+ current that is expressed in human ventricle. This current, denoted I_{Ks}, is distinct from the HERG K^+ current that is illustrated in Fig. 1C.[38,39] Differences include: its activation kinetics are slower, its level of expression is much lower, and it shows conventional (as opposed to anomalous)

dependence upon alterations in the electrochemical gradient for K^+.[39] That is, in this case, when the electrochemical driving force for K^+ increases, (e.g. as a consequence of decrease in $[K^+]_o$ from normal (5.4 or 4.2 mM) to approximately 3 mM), I_{Ks} increases with little or no change in time course or voltage dependence. The four superimposed traces in Panel D of Fig. 1 illustrate the changes in I_{Ks} during the APs shown in Panel A. Note, however, that this current in human ventricle (as opposed to its size in guinea pig ventricle[20]) is only approximately 10% the size of the outward peak current due to I_{K1} (as shown in Panel B). Nevertheless, at increased heart rates, and/or in the setting of enhanced sympathetic tone,[40] I_{Ks} is known to have an important role in the final repolarization phase of the human ventricular AP.

The four superimposed traces in Fig. 1E show the changes in electrogenic current due to the Na^+/K^+ pump during the APs illustrated in Fig. 1A.[41] It is apparent that during the AP, this current change at any of the selected $[K^+]_o$ values is very small, only perhaps 2% of that due to I_{K1}. Nevertheless, a detectable outward current change is generated during the AP at each selected $[K^+]_o$ level. This net outward current plays an essential role in overall K^+ homeostasis of the myocyte and may also alter the repolarization waveform in human ventricle. Note, however, that the changes in this electrogenic or pump-mediated current are due to the alterations in the AP waveform produced by $[K^+]_o$. These depend on the intrinsic voltage-dependence of this enzyme, and the known dependence of its turnover rate on $[K^+]_o$. However, since the affinity or K_d for the external binding site for K^+ on this Na^+/K^+ pump is approximately 2.0-2.5 mM,[42] the physiological changes in $[K^+]_o$, which are the focus of this paper, would *not* be considered to be primary or strong modulators of this small electrogenic current under

relatively short term changes in APD that occur in response to alterations in $[K^+]_o$. In contrast, during maintained periods of enhanced heart rate or extramural stimulation the contribution of this electrogenic Na^+/K^+ pump current may increase. [43,44]

The results in Figs. 1-2 (in context of Figs S-1 and S-2) provide most of the required information that is needed to integrate and then illustrate the electrophysiological consequences of altered $[K^+]_o$ on the AP of baseline or healthy myocytes human ventricle. Both basic science and clinical papers utilize the concept: repolarization reserve in human ventricle to capture and discuss key aspects of action potential repolarization mechanisms and dynamics.[45,46] Repolarization reserve is equivalent to the net outward current during a membrane or non conducted action potential.[47] It is of interest to follow the changes in this parameter (i) during initiation of repolarization from the plateau, (ii) throughout final repolarization and (iii) also in the inter-stimulus interval, that is during diastole. The four superimposed I-V curves in Fig. 3 illustrate the net current in the selected $[K^+]_o$ levels that are the focus of this illustrated review with reference to the four corresponding AP waveforms in Fig. 1A. Two aspects of the net current records in 4.2 and 3.5 mM $[K^+]_o$ are noteworthy: (i) The reduction in $[K^+]_o$ and related shift in the underlying, nonlinear, I-V curve for I_{K1} give rise to a 'flat' trajectory at about 220-250 ms (within the plateau). This is important since it will render this phase of the AP very sensitive to intrinsic (e.g. autonomic transmitter) or extrinsic (stretch and/or applied current) perturbations. (ii) The entire net current I-V relation, i.e. the repolarization reserve capacity, shows anomalous dependence on $[K^+]_o$: reducing $[K^+]_o$ decreases the repolarization reserve in spite of the net current being K^+ selective and the electrochemical driving force for K^+ being increased.

Another demonstration of the practical significance of alterations in plasma $[K^+]_o$ within its physiological range can be illustrated with an analysis of some aspects of the frequency-dependence of AP waveform.[48,49.] In both experimental and clinical settings, this cardiac electrophysiological information can be obtained using two distinct, but related protocols. The first protocol involves measuring AP duration at a fixed level of repolarization (in our case APD_{60}), while introducing a single extra-stimulus in the diastolic period following a stimulation train (S1) at 1 Hz. The resulting data, denoted the interval-duration relation, or restitution curve for repolarization, when obtained at 1 Hz steady-state simulation in either 5.4 or 3.5 mM $[K^+]_o$, is shown in Fig. 2A. In this Figure each data point corresponds to the APD_{60} at a selected inter-stimulus, or S_1-S_2, value. In both Figs. 2A and 2B the data set denoted by black circles was obtained using a $[K^+]_o$ of 5.4 mM, while the data denoted by red triangles was obtained in 3.5 mM $[K^+]_o$. Note that (as expected from Fig. 1) the lengthening in the AP due to reducing $[K^+]_o$ from 5.4 to 3.5 mM results in an upward shift of these I-D relations or restitution curves. This upward shift is regulated by the increased duration of the AP, and the corresponding lengthening of the relative refractory period. The data in Fig. 2B consists of analogous results obtained at a steady-state stimulus frequency of 2.5 Hz. In this case, the lengthening of the AP in 3.5 mM $[K^+]_o$ also results in 2 distinct I-D curve trajectories[48-50]

[Fig. 2 near here]

As mentioned, repolarization reserve is a useful concept for assessing the stability of an electrophysiological substrate. It is also well recognized that the

impedance profile (relative changes in input resistance as a function of the phase of the AP waveform; i.e. plateau, early repolarization, late repolarization and/or in diastole) can strongly modulate not only repolarization but also intercellular communication. Both of these factors contribute to the essential properties of the ventricular syncytium. The classical work of Weidmann first demonstrated that during the plateau of the cardiac (Purkinje fiber) AP, the input resistance was 2.5 - 4.0 times larger than that measured during diastole.[47]

Since changes in $[K^+]_o$ alter both the resting potential and the AP waveform in the ventricle,[13,14,18] we have used an approach developed by Zaniboni et al.[51,52] to calculate relative input resistance at three different phases of the simulated electrophysiological activity in human ventricle in the presence of selected $[K^+]_o$ conditions. These calculations were done and are illustrated in Fig. 3: (i) during diastole, (ii) at the AP plateau and (iii) during final (late) repolarization. As expected (and summarized in Table 1), alterations in $[K^+]_o$ resulted in significant and progressive changes in this profile of phase-specific slope resistances.

With respect to the main focus of this review, note that decreasing $[K^+]_o$ from normal (5.4 or 4.2 mM) to 3.5 mM caused an approximately 10-25% increase in input resistance during diastole. During the plateau (at 0 mV) the input resistance in low $[K^+]_o$ increases 5-10 fold; and in final repolarization (-40 mV) the myocyte also exhibited enhanced (30-50%) input resistance. These changes would be expected to reduce the electrophysiological stability of the human ventricular myocyte during diastole. They would also make the preparation (myocyte or trabeculum) more sensitive to external stimuli during the plateau. Note also that these changes can be detected during

approximately 25-30% of the electrophysiological duty cycle that corresponds to each heartbeat.

[Fig. 3 and Table 1 near here]

It has previously been demonstrated that superfusion with reduced $[K^+]_o$ and/or reductions in I_{K1} (or HERG) may result in an increased incidence of early after-depolarizations (EADs) in mammalian ventricle.[53-56] Accordingly, we have explored: i) changes in HERG and/or I_{K1} that can result in EAD generation and also ii) determined whether co-incident reductions in $[K^+]_o$ could be considered to be an additional pro-arrhythmic factor or 'double hit'. Our choice of the ORd model[34] as a platform for this illustrated review and the decision to modify it (as explained in conjunction with Figs. S1 and S2) was based in part on the previously demonstrated capability of this model to mathematically simulate EADs in both single isolated human ventricular myocytes; and also in multi-cellular strands (trabeculae) or ventricular transmural wedge preparations.

The three superimposed traces in Fig. 4 an interesting and informative pattern of *in silico* responses. In all cases, the experimental conditions of Guo et al.[53] are replicated, so that a defined substrate for EAD generation could be used as a starting point. Specifically, this human ventricular myocyte was driven at a low rate (0.25 Hz) in normal $[K^+]_o$ (5.4 or 4.2 mM) and HERG was reduced significantly (by 70%). As expected, the AP lengthened substantially, and in 4.2 mM $[K^+]_o$ there was a hyperpolarization of the resting potential. A second set of simulations were done under these same conditions except that $[K^+]_o$ was reduced from 4.2 to 3.5 mM. The results

revealed not only hyperpolarization of the resting potential but also EAD formation near the end of the AP plateau.

Note, (as shown in Fig. 3) that reduction in $[K^+]_o$ would be expected to alter/enhance intercellular coupling due to the $[K^+]_o$-sensitive change in impedance profile at depolarized membrane potentials. In principle, this could result in the coupled myocytes exhibiting an enhanced pro-arrhythmic profile due to increased EAD activity that could propagate to neighboring myocytes. It is also likely that the changes in cell-to-cell transcellular electrotonic currents could contribute to *de novo* EAD generation in 'downstream' myocytes in the ventricular myocardium.

[Fig. 4 near here]

Important principles and functional aspects of the effects of low $[K^+]_o$ on the transmural conduction and electrophysiological stability/responsiveness of the human ventricular action potential are illustrated in the Supplementary Section (Fig. S-3). These simulations and the resulting mechanistic insights concerning ventricular substrate excitability and stability are similar to those revealed previously by mathematical simulations of the guinea pig ventricle myocyte action potential.[20]Both of these sets of simulations suggest that particularly in settings of low heart rate, the abnormally long diastolic intervals contribute to pro-arrhythmic electrophysiological 'triggers' can emerge partially in response to direct and secondary consequences of low $[K^+]_o$ conditions.

New Insights and Perspectives

This illustrated review demonstrates that key components and principles of human ventricular electrophysiology are sensitive to even very small changes in plasma $[K^+]_o$ levels and provides new insights into the underlying ionic targets and mechanisms. Some aspects of the effects of low $[K^+]_o$ on human ventricle have previously been studied in detail.[11,20,21,44,57,58] However, in one such paper, $[K^+]_o$ was reduced very substantially, to less than 1mM.[57] Under these conditions one would expect the marked changes in K^+ channel function to also be accompanied by strong inhibition of the Na^+/K^+ pump.[41-43] In contrast, the much smaller range of $[K^+]_o$ changes selected as a basis for this review often occur as a consequence of normal healthy living activities e.g., exercise paradigms.[2,3] These conditions may also result from necessary medical treatments, such as diuretic therapy.[6,7] Recent direct measurements of plasma $[K^+]_o$ activity yield a mean value of approximately 4.2 mM in a healthy adult.[11,58]

One consequence of the marked increase in duration of the AP resulting from reduced $[K^+]_o$ is secondary and significantly altered $[Ca^{2+}]_i$ homeostasis.[59,60] It is well known that the peak of the $[Ca^{2+}]_i$ transient elicited by the AP at a fixed heart rate increases somewhat, and its duration also lengthens, when $[K^+]_o$ is decreased. Both of these changes would be expected to result in a positive inotropic effect, and indeed this has been reported in both guinea pig and rabbit heart preparations.[8,61,62]

Cellular Mechanisms Regulating Low $[K^+]_o$ Effects

Our semi-quantitative analyses of the action potential 'phase specific' input resistance of the human ventricular AP [51,63,64] that occurs in the setting of low $[K^+]_o$ also

revealed significant changes, (as shown in Fig. 3). These changes would be expected to alter the repolarization reserve [38], and also to indirectly but significantly modulate intercellular coupling. [18,47] As we have illustrated, a decrease in $[K^+]_o$ from approximately 4.2 – 3.5 mM caused an approx. 3-fold increase in the membrane resistance at the resting potential. Perhaps more importantly, (and as a consequence of the cross-over of the ion-transfer or I-V relationships, see Fig S-2), in response to $[K^+]_o$ changing the resistance at the plateau level of the AP can increase very significantly, (approximately 8-10 fold) . Both of these changes result in the myocyte (and indeed a system of connexin-coupled myocytes) being much more sensitive to external perturbations e.g., stimulus currents, autonomic or ligand-gated ion channel activation, stretch. This increase in myocyte-to-myocyte coupling may also increase conduction velocity and improve contraction synchronicity. Moreover, it would be expected to also provide an increased tendency/capability for abnormal impulses (e.g. EADs or DADs) to alter 'distant' AP waveforms. Thus, low $[K^+]_o$ may contribute to a pro-arrhythmic substrate.[20,44,45] The simulations in Figs. 4 and Fig. S-3 illustrate how that this can occur, even in healthy human ventricle.

Our findings highlight the importance of K_{ir} 2.1 or perhaps 2.2 channels that generate an inwardly rectifying, time-independent or background current in ventricular myocytes. It is important, therefore, to recall that a substantial fraction of the cellular expression of this type of K^+ channel is localized in the T-tubule system.[65,66] Recognition of this, combined with recent reports demonstrating that in the setting of heart failure (HF) the transverse tubular system undergoes significant decrease,[66] may explain the tendency of the human ventricle in the HF setting to be unstable at, or near, its resting

or diastolic range of membrane voltages. This instability, due to a significant reduction in the outward component of I_{K1}, may reveal or unmask pro-arrhythmic tendencies of natural products or prescribed drugs that are otherwise considered both safe and efficacious even when administered to patient populations with apparently unrelated chronic diseases in addition to the setting of heart failure.[60,67]

Effects of $[K^+]_o$ on the Relative Refractory Period and Restitution of AP Duration

It is well known that changes in $[K^+]_o$ can alter electrophysiological restitution in mammalian ventricles.[13,20] Perhaps the most straight forward aspect of this is that changes in excitability and relative refractoriness are due to the $[K^+]_o$-dependent alterations in resting potential. The predominant effects of low $[K^+]_o$, a significant hyperpolarization would be expected to reduce Na^+ channel inactivation, and thus augment excitability and the associated supernormal period. This effect is well-established, as judged either in terms of action potential threshold or conduction velocity.[20,67,68] Restitution of AP duration is also modulated by $[K^+]_o$ levels. The prevalent (classical) explanation for this is that $[K^+]_o$-dependent changes in the relevant K^+ currents: I_{K1}, I_{Ks}, and I_{Kr} (HERG) alter (i) resting potential and/or (ii) AP duration at a fixed stimulus or heart-rate. The size of I_{K1} and the deactivation kinetics of HERG and I_{Ks} modulate the initial slope and the overall trajectory of this interval-duration or restitution curve/relationship.[51,52]

Translational Significance of Low $[K^+]_o$ Effects

Structure-function aspects of projects in Medicinal Chemistry and the Safety Pharmaceutical Groups in industry and in governments may need to consider and integrate the marked, often nonlinear effects that even small changes in plasma $[K^+]_o$ levels can have on the standard and well-accepted 'lumped parameters' that are used as standard criteria/end points.[69,70] For example, 'efficacy' as judged by concentration-response relationships; and safety profiles for pro-arrhythmic tendencies (as monitored by APD measurements on rate-corrected Q-T data) may not be able to be attributed entirely and mainly to changes in HERG. These parameters may change significantly as a consequence of activity, drug (diuretic therapy) or diet-induced alterations in plasma electrolytes. It is beginning to be recognized that developing more robust criteria for Drug Safety will need to include a battery of coincident effects of drug candidates on plausible ion channel targets.[71-74] This constructive change in approach and perhaps in policy needs to include detailed consideration of plasma $[K^+]_o$ levels.

Perhaps the most obvious application of our findings to Clinical Pharmacology/Therapeutics would be patient groups that are receiving diuretic therapy in conjunction with drugs administered with the goal of achieving improved rate or rhythm control. While it is acknowledged that this group will be heterogeneous, it may be that consideration of possible contributions of plasma $[K^+]_o$ to (i) pro-arrhythmic incidence, or (ii) related e.g. muscle twitches or camping worthwhile as one criterion of drug efficacy and related tolerance/compliance.

It is well-known from clinical studies of the relationship between $[K^+]_o$ and mortality that *either* low or high $[K^+]_o$ can increase mortality rates.[75-76] The principles and

illustrations in this review provide insights into that finding: both high and low K^+ depolarize human ventricular tissue (and Purkinje fibres) while also markedly changing the action potential waveform and substrate impedance (input resistance 'along' the action potential plateau).

Authors Contributions

Doctors Trenor, Saiz and Giles shared responsibilities for study design. Computations were performed at Universitat Politècnica de València by Doctors Cardona and Trenor, Dr. Giles wrote this manuscript. All authors contributed to final editing.

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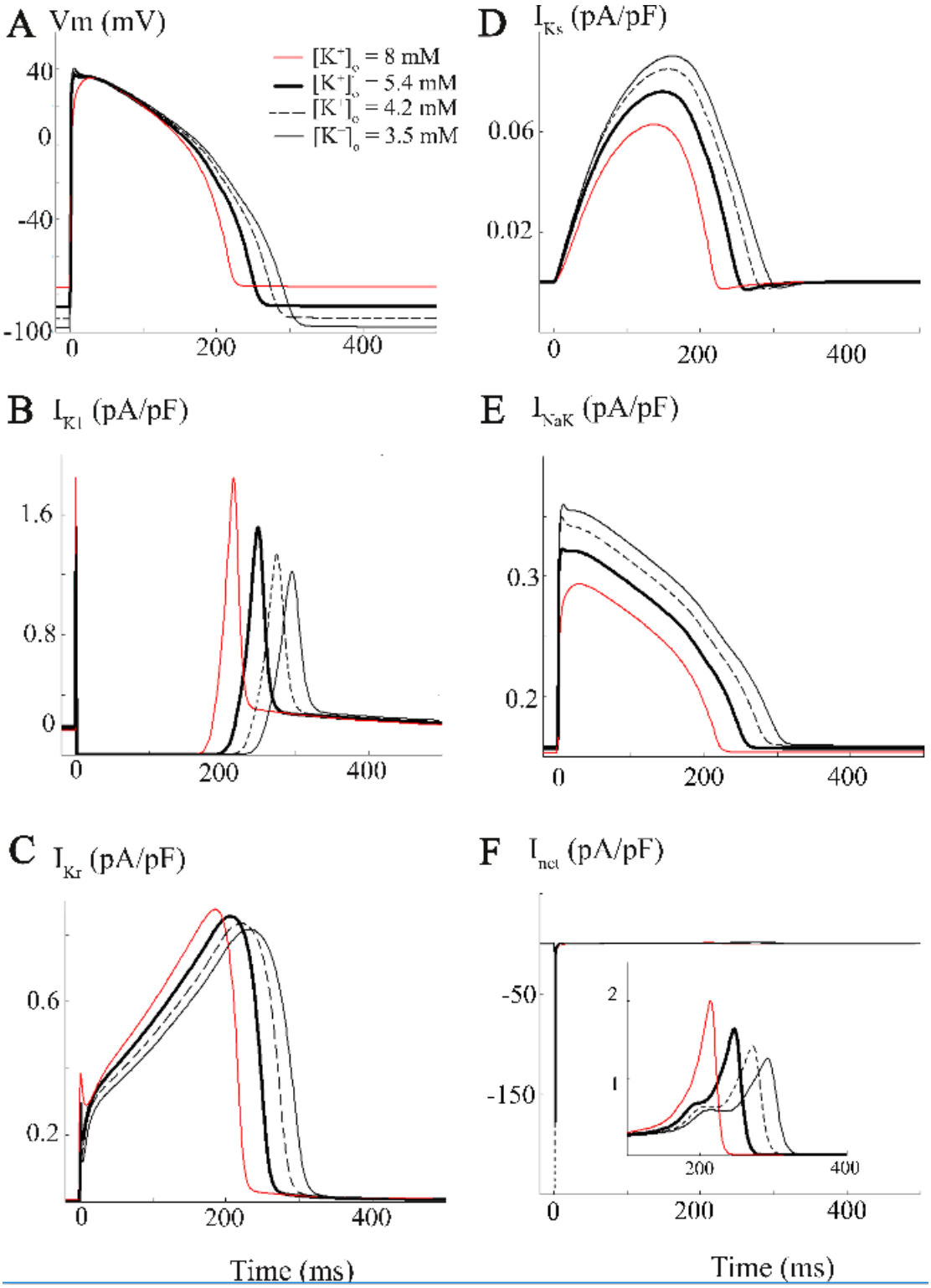

Figure 1. Illustration of the responses of the delayed rectifier K^+ currents (HERG and I_{Ks}) and Na^+/K^+ pump current in human ventricle to the changes in $[K+]_o$ chosen for this

study. Panel A shows four superimposed action potentials (APs) at steady state (1 Hz) in response to changes in $[K+]_o$. These simulations were done using our modified ORd model of the human ventricular myocyte AP. That is, the mathematical expressions for I_{K1} from the GBP model were substituted for those in the published ORd and the changes described in the Methods section were introduced. Note the marked changes in resting potential, together with altered AP durations at all membrane potentials negative to 0 mV. Panel B shows the alterations in I_{K1} produced by these changes in $[K^+]_o$. The data in Panel C illustrate the analogous responses of HERG to changes in$[K^+]_o$. As expected from experimental findings and clinical data sets the decrease $[K^+]_o$ from the normal range, either 5.4 mM (experimental) or 4.2 mM (human plasma K^+ levels) reduce the current and lengthen the AP, in spite of the fact that the electrochemical driving force for K^+ is increased by this maneuver, namely decreasing $[K^+]_o$ to 3.5 mM. Note (Panel D) that the response of I_{Ks} to a decrease in $[K+]_o$ is conventional: that is, as the driving force is increased by the simulated decrease in $[K+]_o$, peak current increases. Panel E in this Figure shows the electrogenic current generated by the Na^+/K^+ pump. In both the ORd and GPB models, this current exhibits intrinsic voltage-dependence, as indicated by the fact that it 'scales' as the alterations in $[K^+]_o$ change the resting potential and/or the height or duration of the AP. For these reasons, this current increases in low $[K^+]_o$. Note, that both I_{Ks} and I_P are much smaller (approximately 10x) than either I_{K1} or HERG. See Results and Discussion for further explanation. Panel F shows the corresponding net currents (calculated using the method developed by Zaniboni.[63,64] Note that reducing $[K^+]_o$ decreases the net current corresponding to both the early and late repolarization phases of the AP. Thus, 'repolarization reserve' is reduced.

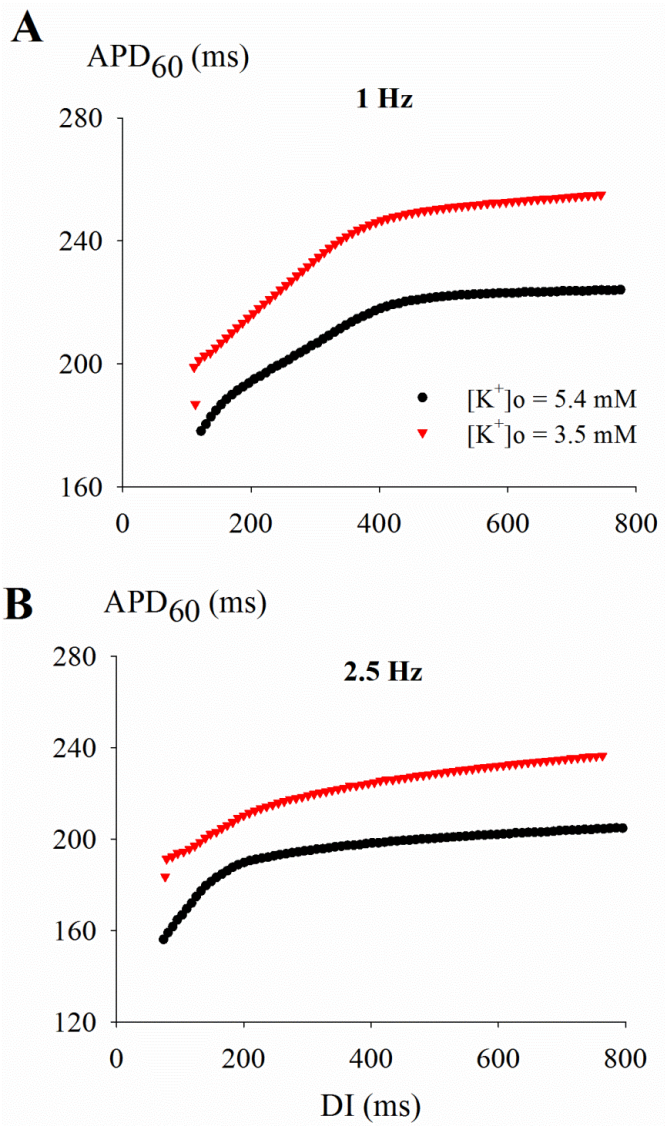

Figure 2. Illustration of the effects of reductions in $[K^+]_o$ on the action potential (AP) durations at 1 Hz (Panel A) and 2.5 Hz (Panel B). These results are presented as restitution curves, i.e. the APD in response to a single extra-stimulus (S_2) measured at APD_{60}, the 60% repolarization level of the AP. For these simulations, our modified ORd model was used. Both the patterns of results are conventional: AP duration shortens as the diastolic interval deceases. The increase in AP duration in response to the decrease to 3.5 mM in $[K^+]_o$ results in the restitution curve shifting in the upward direction by an amount that corresponds closely with the change in AP duration.

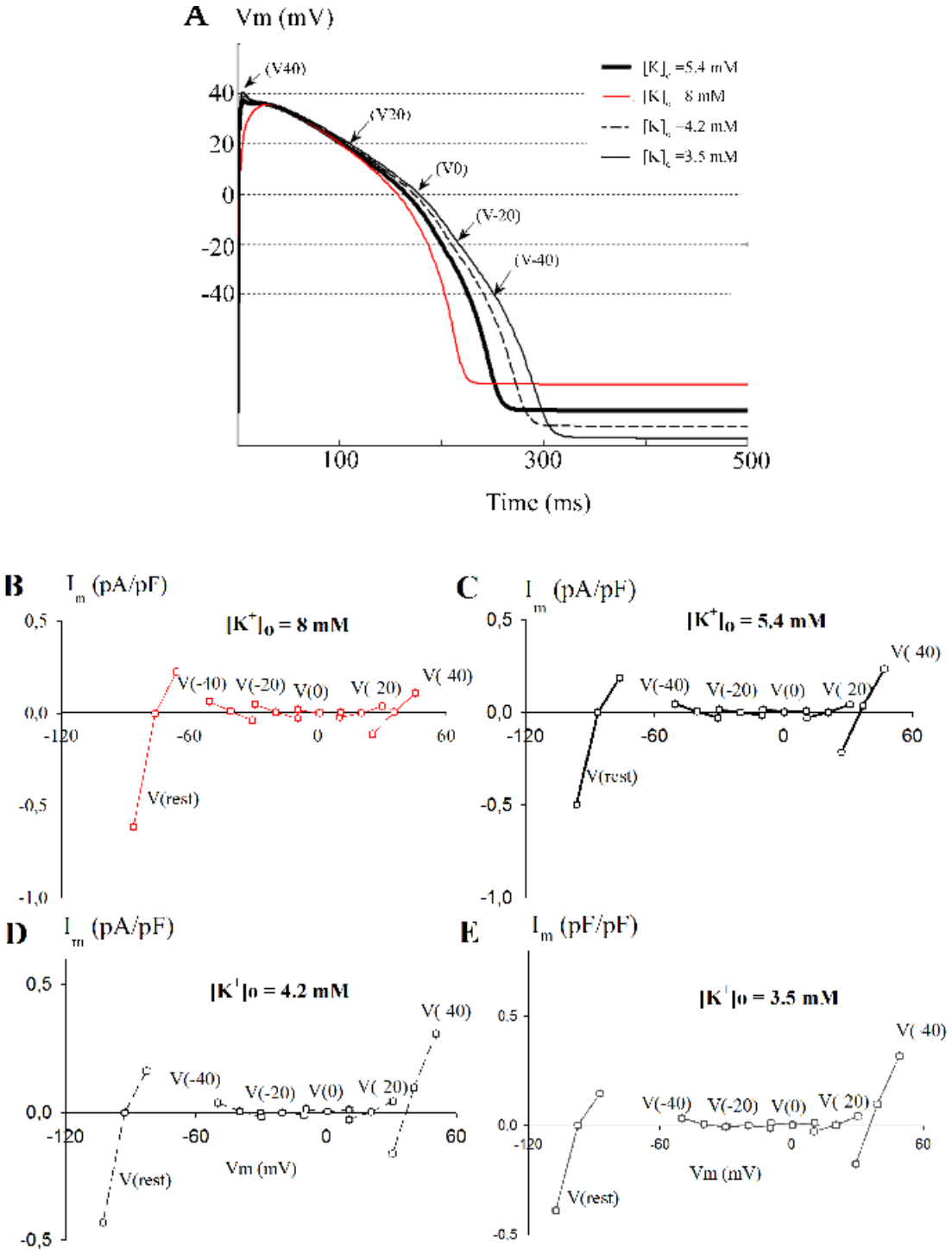
A
Vm (mV)
(V40)
(V20)
(V0)
(V-20)
(V-40)
[K]o =5.4 mM
[K]o 8 mM
[K]o =4.2 mM
[K]o =3.5 mM
40
20
0
-20
-40
100
300
500
Time (ms)
B
Im (pA/pF)
[K+]o = 8 mM
V(-40) V(-20) V(0) V(20) V(40)
V(rest)
C
Im (pA/pF)
[K+]o = 5,4 mM
V(-40) V(-20) V(0) V(20) V(40)
V(rest)
D
Im (pA/pF)
[K+]o = 4.2 mM
V(-40) V(-20) V(0) V(20) V(40)
V(rest)
Vm (mV)
E
Im (pF/pF)
[K+]o = 3.5 mM
V(-40) V(-20) V(0) V(20) V(40)
V(rest)
Vm (mV)

Figure 3. Calculation of the changes in input (slope) resistance within defined ranges of membrane potential in control (5.4 mM, experimental; or 4.2 mM clinical) $[K^+]_o$ conditions, and also in high (8mM) and low (3.5 mM) $[K^+]_o$ during the action potential (AP) in a human ventricular myocyte. The arrows in Panel A illustrate the data collection points for each of the 4 superimposed AP (1 Hz, steady-state) waveforms. Panels B-E show the slope resistance in the defined ranges of membrane potential. The corresponding numerical data are summarized in Table 1. Note that in low $[K^+]_o$ (3.5 mM), there is a significant increase in input resistance (i) at resting potential; and (ii) at membrane potentials corresponding to the AP plateau. Action potentials (APs) were computed using the modified O'Hara et al. model (ORd_m) assuming $[K^+]_o$ (8, 5.4, 4.2, and 3.5 mM), respectively. The arrows indicate the membrane voltage levels (V_m) for which I-V curves were constructed. Panels B, C, D, and E. Total membrane I-V curves for $[K^+]_o$ of 8, 5.4, 4.2, and 3.5 mM, respectively, computed as in Zaniboni 2011 and 2012 [63, 64] (see Methods in Supplementary material).

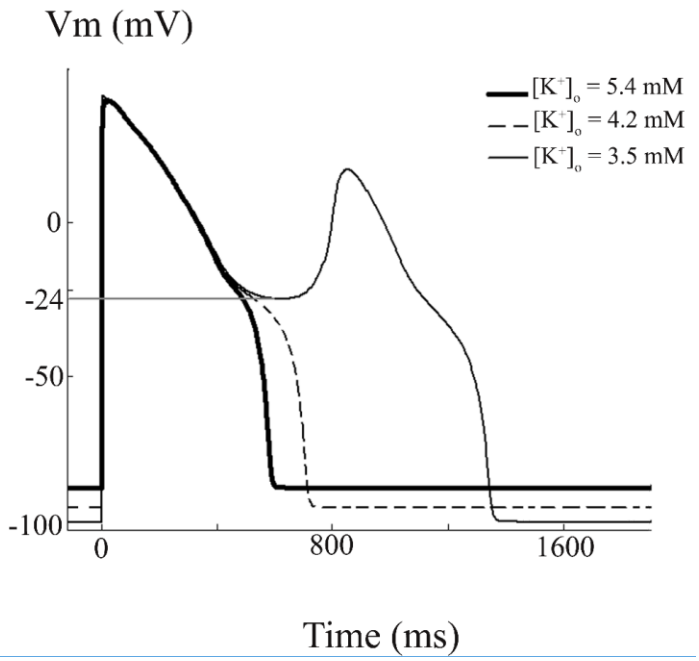

Figure 4. In low $[K^+]_o$, early after-depolarizations (EADs) may develop at the plateau level of the action potential (AP) in human ventricular myocyte. Three simulations were performed using our modified O'Hara et al. model (ORd_m), assuming 5.4, 4.2, or 3.5 mM $[K+]_o$. The stimulus basic cycle length (BCL) was 4000 ms, or 0.25 Hz. Note that EADs were generated only when $[K^+]_o$ was 3.5 mM. The membrane potential (V_m) from which EADs develop is indicated by the discontinuous horizontal line. (See text for further description of initial conditions for these simulations.)

Membrane Resistance Changes as a Function of $[K^+]_o$ and Phase of the Action Potential

V_m(mV)	$[K]_o$ = 8 mM	$[K]_o$ = 5.4 mM	$[K]_o$ = 4.2 mM	$[K]_o$ = 3.5mM
	R_m MΩ	R_m MΩ	R_m MΩ	R_m MΩ
40	90	44	43	40
20	333	290	282	284
0	-1465	-2922	-10852	22626
-20	-269	-711	-2132	-3554
-40	-198	-269	-361	-495
V(rest)	24	29	34	37

Table 1. Membrane resistance (R_m, Megaohms) calculated as the inverse of the slope of the instantaneous I-V curves shown in Figure 7 (panels B, C, D, and E). R_m was calculated at defined points on the I-V curves. $[K^+]_o$ values of 8, 5.4, 4.2, and 3.5 mM were studied.

Supplemental Material

Mathematical models of the human ventricular action potential

To succinctly convey the main principles that are the basis of this Review, mathematical simulations of the electrical activity of endocardial human ventricular myocytes were done using the two most comprehensive published action potential (AP) models: Grandi et al. (GPB),[1] and O'Hara et al. (ORd).[2] The GPB model is based on detailed semi-quantitative descriptions of intracellular calcium homeostasis, as well as the main ionic currents, derived from a subset of published human ventricle data. It has proven to be a useful tool for simulating the normal AP and related repolarization abnormalities.

The ORd model[2] originates from the extensive rigorous work of the Rudy Group on guinea pig ventricular myocytes[3]. Importantly, however, it is based on experimental data taken from 140 healthy human hearts. This model includes detailed mathematical formulations for 18 ionic currents and also provides the possibility of computing AP waveforms for phenotypic subsets of heart cells: endocardial, epicardial, and M myocytes. The inclusion of these transmural electrophysiological heterogeneities in the ORd model is an important capability. This model also provides the possibility of carrying out 1-dimensional transmural 'ventricular wedge' simulations and these can be used to explore the basis for changes in pseudo-electrocardiograms (ECGs) as shown in Figure S3.

Computational methods

The GPB and ORd model differential equations were both implemented in Matlab (Math-works Inc., Natick, MA, USA) and then solved numerically using a variable order solver (ode15s) as we have done in previous published work.[4] All model equations and code were taken from O'Hara et al.[2] which was downloaded from http://rudylab.wustl.edu, and 1D simulations were run using Microsoft Visual C++.

Validation and modification of these action potential models for simulations of hypokalemia

As a first step in developing illustrative material for this Review the effects of changes in $[K^+]_o$ on the human ventricular AP were compared using GPB and ORd models. Figure S-1 shows the marked prolongation (or shortening) of the AP under simulated hypokalemic ($[K^+]_o$=3.5 mM)[5,6] and hyperkalemic ($[K^+]_o$=8 mM) conditions, respectively. For both models, the baseline or control $[K^+]_o$ was set at 5.4 mM: the value is most common in experimental studies.

Somewhat to our surprise, the simulated action potentials and changes in resting potential produced by these two models of the human ventricular myocyte differed very significantly when $[K^+]_o$ was altered. Specifically, there was an *increase* of the inward-rectifying K^+ current (I_{K1}) when $[K^+]_o$ was *decreased* to 2.5 mM using the ORd model (Figure S-1D). In contrast, I_{K1} current density *decreased* quite markedly in the GPB model (Figure S-1C) in response to this maneuver. Since it is well known that I_{K1} *decreases* under hypokalemic conditions,[3,7-11] this difference needed to be understood fully.

[Figure S1 near here]

Figure S-2A shows simulated I-V curves for I_{K1} using the published ORd model[2]. This was done in an attempt to reproduce the relevant patterns of published experimental results such as those from our previous paper[9] that are illustrated in the inset of this Figure. Unfortunately, an *incorrect increase* in I_{K1} in low $[K^+]_o$ was observed. In contrast, and as noted, when the GPB[1] formulation of I_{K1} was used to simulate low $[K^+]_o$ (Fig. S-2B), this model quite accurately reproduced the experimental observations.

We therefore gave serious consideration to utilizing only the GPB model for the illustrations in this review. However, the ORd model is based on an impressive cross-section of experimental data from human ventricle; and it provides the possibility of examining the effects of changes in $[K^+]_o$ on transmural AP propagation and the pseudo ECG. In addition, the ORd model has been selected for the Systems Biology component of this ongoing CiPA initiative that is expected to result in changed Safety Pharmacology Guidelines for anti-arrhythmic drug assessments.[12]

We therefore modified the published ORd model[2] so that it could accurately replicate the well-known responses to altering $[K^+]_o$. This was done by introducing the I_{K1} formulation from GPB into the ORd AP model, i.e., replacing the original ORd I_{K1} equations. We also noted that the resting potential (V_{rest}) in control conditions in the ORd model is much more hyperpolarized (V_{rest} = -88 mV) than in the GPB model (V_{rest} = -81 mV). As a consequence, in the ORd model V_{rest} and E_k are identical, thus the I_{K1} value at rest was zero. Accordingly, for all our simulations V_{rest} was adjusted to -86.5

mV in the ORd model. This was achieved by: (i) decreasing the Na^+/K^+ pump current (I_{NaK}) density by 30%, (ii) introducing the GPB formulation for the background Na^+ current (I_{Nab}). This results in a 3-fold increase in this current density and (iii) decreasing the background K^+ current (I_{Kb}) by 50%. The resulting behavior of the modified ORd model (denoted ORd_m) for selected $[K^+]_o$ levels is shown in Figure S-1.

[Figure S-2 near here]

In silico stimulation and analysis protocols

Ventricular myocytes were stimulated *in silico* at 1Hz (unless otherwise specified) until steady-state conditions were achieved. To model the restitution curves for AP duration (APD) shown in text or main body of this paper as Fig. 2, each APD was measured at 60% repolarization (APD_{60}). Specifically, ventricular myocytes were subjected to a 1 Hz or 2.5 Hz stimulus train, until steady-state conditions were achieved. A single extra-stimulus was then applied at each selected coupling interval, ranging from approximately 1000 ms to 230 ms. These intervals varied somewhat depending upon the chosen steady-state stimulation frequency (either 1 Hz or 2.5 Hz). In practice, the AP lengthening caused by simulated effects of low $[K^+]_o$ meant that in 3.5 mM $[K^+]_o$ the smallest inter-stimulus interval was 260 ms. The APD_{60} values corresponding to the selected S2 stimuli were plotted vs. the diastolic interval as shown in Figure S-2A, B[13-15] as a conventional APD restitution relationship.

Membrane resistance computations

The membrane resistance (R_m) was calculated using the approach developed by Zaniboni[16,17]. In brief, the total membrane current (I_m) was sampled and divided by the membrane voltage (V_m), at the selected points within the small voltage range (V_{mx}) indicated by arrows in text Figure 3A. Myocytes were stimulated at 1 Hz. After achieving steady-state conditions, each simulation was interrupted at a chosen time during the AP plateau or repolarization. These times are denoted V_x in Figure 3A. At each the total ionic current (I_{ionx}) was measured and the capacitive current (I_C) was calculated. V_m was then immediately maintained constant or voltage clamped for 10 msec at the selected values denoted: V_x, V_x–10 mV, or V_x+10 mV. In Figure S-3, the sympol x denotes the time at which the resistance was calculated. I_m was computed as the total ionic current measured at 1 ms after the onset of the voltage clamp (I_{ion}). From this data I_m-V_m curves were constructed and R_m was calculated as the reciprocal of the slope of these curves.

Simulations of 1-dimensional conduction using the ORd transmural wedge model

In these simulations (illustrated in Fig. S-3A and B), our modified ORd model of the human ventricular AP was utilized together with a heterogeneous substrate consisting of a 1-dimensional strand of 165 myocytes that were coupled end-to-end. A train of 700 suprathreshold stimuli was applied at 1 Hz the extreme endocardial of this strand or *in silico* wedge preparation. After steady-state was achieved, measurements of AP waveform were recorded as in O'Hara et al.[2] The resulting AP propagated in the 'inside-out' direction, thus replicating some aspects of transmural activation and

conduction in the human left ventricle. To enhance the probability of EAD generation, a number of changes in the baseline parameter set of the ORd model were introduced. Each of these was selected to align with published information concerning arrhythmia (EAD) induction in human ventricles.[18-22] The control (5.4 mM) electrophysiological waveforms in either 5.4 mM or 3.5 mM $[K^+]_o$ at a steady rate of 0.25 Hz, are shown in Panels A and B, respectively. In each panel the selected colors illustrate the AP waveforms at fixed positions denoted by cell number: (i) blue, cell #32, endocardium; (ii) red, cell #65, M cell region; (iii) green, cell #98, M cell region; and (iv) magenta, cell #132, epicardium. This somewhat simplified initial analysis, shows that in low $[K^+]_o$ the EAD complex can move from myocyte to myocyte more readily[23-27] mainly due to the altered ratio of myocyte input resistance to intercellular/atrial resistance arising from the K^+ induced nonlinear changes in I_{K1}.

[Fig. S-3 near here]

Limitations and opportunities for additional studies

This review provides current information concerning the marked sensitivity of human ventricular electrophysiology to $[K^+]_o$, with emphasis on the effects of low but clinically relevant $[K^+]_o$ levels, approx. 4 mM.[28,29] Our illustrations are based upon a modified (corrected) computational platform for some Systems Biology components of multidisciplinary studies of human ventricle electrophysiology and pharmacology/drug discovery. We acknowledge, however, that our work has some significant limitations.

1. We have focused on the electrophysiological effects of changes in physiological levels of $[K^+]_o$ in isolated human ventricular myocytes. However, our insights may apply mainly to the endocardium of the left ventricle, since only this 'module' of the published O'Hara model[2] was used. Major changes were observed in the resting potential and late repolarization, both in the parent model and after necessary corrections were introduced. Although the underlying ionic mechanisms for these two phases for the duty cycle of cardiac electrophysiological activity[30] are somewhat different in the epicardium and in in the endocardium, many of our illustrations and related insights are expected to apply to the healthy ventricular myocardium *per se*[30]. We recognize, however, that the wave-shape of the AP, and indeed the basis for the transmural electrophysiological heterogeneity, results from small changes in net currents within selective voltage ranges of the action potential.[27, 31-33] Thus, additional work may need to be done, in which the epicardium and endocardium APs are compared.
2. It was necessary to formulate a substantially revised hybrid model of the human ventricular AP. Our initial analysis revealed that the ORd model has only very limited ability to maintain excitability (initiated action potential) in any setting involving resting membrane potential depolarization. Perhaps more importantly, the mathematical formulation for the inwardly rectifying background K^+ current in the ORd model cannot reconstruct or simulate the fundamental and well-known response of the I_{K1} current system, to either increases or decreases in $[K^+]_o$. In principle, there was the possibility of using the GPB model for all of the

calculations in this paper. However, we have encountered significant practical difficulties in introducing the GPB model into the linear cable or transmural wedge preparation, and therefore made use only of the modified ORd model.

3. This study draws attention to, but does not treat in any comprehensive way, how changes in $[K^+]_o$ can modulate intercellular coupling. We demonstrate that even small changes in $[K^+]_o$ can alter the input resistance of the myocytes subjected to low $[K^+]_o$, and we confirm that in cases where the input resistance at the plateau level of the AP increases significantly (See Table 1) these myocytes become more effective current sources provided that intercellular resistance remains constant. This small change is functionally important in a number of plausible pathophysiological settings: it results in a change in 'substrate' that enhances the likelihood of EAD generation and cell-to-cell movement/propagation.[34,35]
4. It will be apparent that the scope of this project was such that a number of important features of the heterogeneity of the human ventricular myocardium are not fully (or even adequately) accounted for. Thus, we have assumed that the selected changes in $[K^+]_o$ would be homogenous throughout the ventricular myocardium. This is somewhat unlikely. Our interpretation assumes that the K^+ channels that sense alterations in $[K^+]_o$ are expressed exclusively in ventricular myocytes. This is known to be incorrect. K_{iv} 2.1/2.2 channels are expressed in endothelial cells, vascular pericytes, and smooth muscle myocytes from 'resistance' vessels/arterioles,[36] changes in $[K^+]_o$ would therefore alter transmural ventricular perfusion profiles. I_{K1} currents are also expressed in ventricular fibroblasts and myofibroblasts.[37-39] Modulation of this current in these non-

myocytes as a consequence of changes in $[K^+]_o$ would be expected to alter ventricular myocyte AP and/or resting potential waveform, due to connexin-mediated electrotonic current flow.[27]

5. Our computations and analyses concerning the interval-duration relationships or restitution curves for APD_{60} (Fig. 2) provide insight into the underlying changes in K^+ currents that are major contributors. We recognize, however, that re-activation of $I_{Ca\text{-}L}$ is also an important contributor.[27] We are also aware that in human hearts electrophysiological restitution (whether measured in terms of conduction velocity or APD) is measurement site specific and often non-monotonic. The reasons involve a contribution from the time-dependent reactivation of a transient-outward K^+ current. However, this interesting principle [27] was not the focus of our study.
6. It is well-known that the ventricular myocardium, as opposed to the isolated single ventricular myocyte reacts to imposed changes in stimulus or heart rate, or alterations in plasma electrolyte content with two distinct time courses.[15] The faster of these takes place in a time window of approx. 30 sec; in contrast, important slower adaptation in action potential waveform and contractility also take place and these develop over 1-10 minutes after the rate or electrolyte changes. The Eisner Group[15] has studied these slower results in detail an attributed them to a combination of key ion fluxes (e.g. K^+) taking place in an environment of restricted diffusion and related changes in Na^+/K^+ pump activity. Perhaps for this reason a number of recent reviews of K^+ dependent alterations of atrial or ventricular electrophysiological activity have strongly emphasized electrogenic pump contribution.[40,41]

7. We recognize also that in clinical settings there are significant and quite wide variations in substrate responsiveness to changes in electrolytes and/or changes in drug regimens. In our initial attempt to illustrate the effects of hypokalemia on fundamental electrophysiological properties of the human myocardium, individual variations and hence Precision Medicine has not been addressed.[42-44]
8. Within the last decade the overall capacity of myocardial cells has been described in terms of their repolarization reserve.[27, 45-47] Although this concept is meaningful and often useful it needs to be utilized and applied selectively and with caution when critical components of it, that is significant parts of the net current that initiates and then drives repolarization consists of nonlinear currents such as the two that are the focus of this review, I_{K-1} and HERG.[48] We have dealt with this interesting point in our recent review on the dynamics of early repolarization.[27]
9. A final significant limitation of the Illustrated Review concerns the extent to which the underlying principles have been set out sufficiently with regard to (i) previous experimental work, and (ii) very recent translational findings. With regard to the former we acknowledge the classical work from the Moe laboratory [48] and related publications from the Jalife Group [49,50] that were among the first to bring out the significance of electrotonic modulation of pacemaker activity, excitability conduction patterns and velocity, and relate these to $[K^+]_o$ dependent changes in the background K^+ current, $I_{K1.}$ Very recent publications that relate to the main principles of this review include: (a) the recognition that both hypo- and hyperkalemia need to be accounted for in the treatment management of

hypertension nad diastolic dysfunction [51,52]; (b) emerging data sets that advance our understanding of the molecular basis for the interactions of $[K^+]_o$ with defining ion channel or ion channel complex components, and (c) the indication that during sleep $[K^+]_o$ may change significantly.

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Figures and Legends

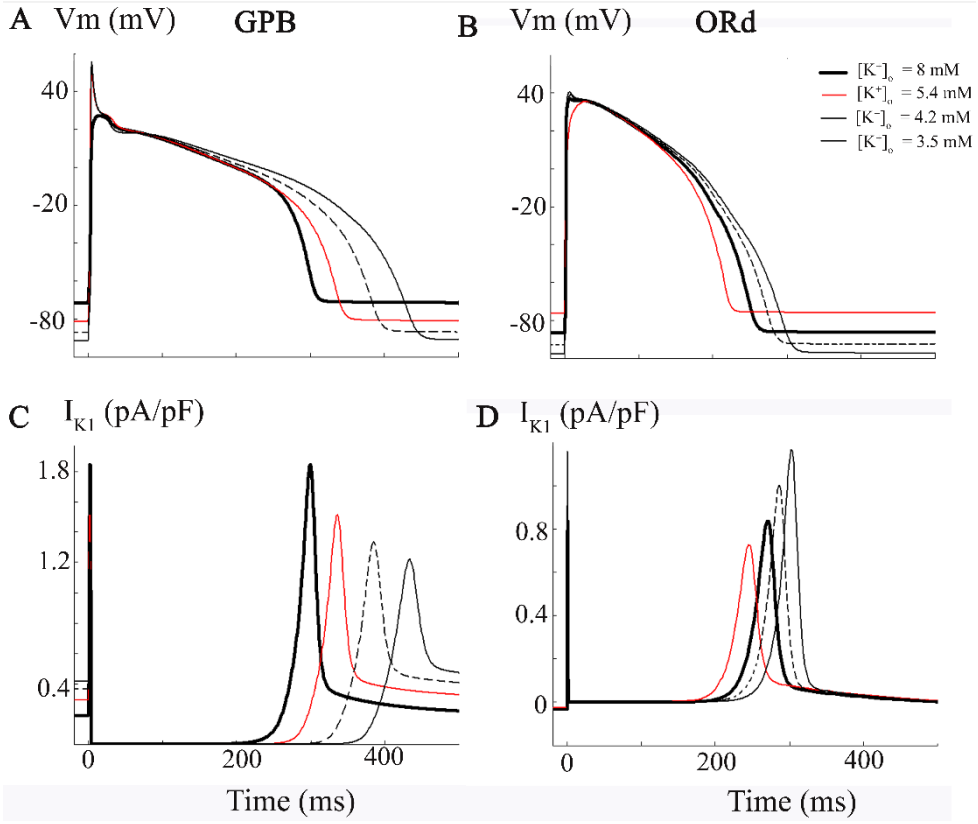

Figure S-1 Response of the inwardly rectifying K^+ current, I_{K1}, in a human ventricular myocyte to selected changes in $[K^+]_o$. Panels A and B show the changes in resting potential and in action potential (AP) waveform in response to the four $[K^+]_o$ values listed in the upper right-hand corner. The 4 superimposed APs in Panel A were computed using the Grandi et al. (GPB) model, while the four on the right are from the O'Hara et al. (ORd) model of human (endocardial) ventricular myocyte. The corresponding data sets in Panels C and D show the underlying changes in I_{K1} during the AP in response to these selected alterations in $[K^+]_o$. Note, that the GPB model correctly simulates the anomalous decrease in peak I_{K1} when $[K^+]_o$ is decreased, while in the ORd model an incorrect pattern of results is obtained: namely, I_{K1} increases when $[K^+]_o$ is decreased. In this and all subsequent figures, the two $[K^+]_o$ that approximate the normal range for

experimental work (5.4 mM) and the mean value for plasma K^+ activity (4.2 mM) are denoted by the thick black and dashed black traces, respectively. Physiological increases in $[K^+]_o$ to 8 mM are indicated by the red traces, while low levels of $[K^+]_o$ (3.5 mM) are illustrated by the thin black traces.

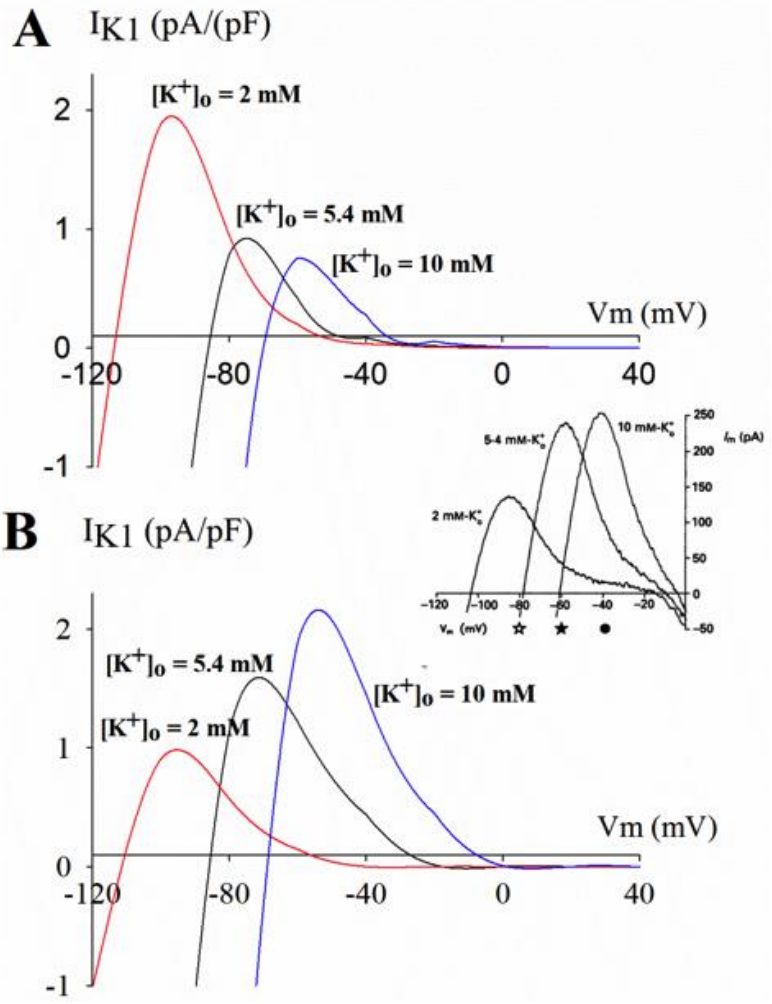

Figure S-2. Illustrations of the marked changes in size and voltage-dependence of the nonlinear I-V curve for I_{K1} computed using the original O'Hara et al. model (ORd) (panel A) versus the Grandi et al. model (GPB) (panel B), for $[K^+]_o$ of 2, 5.4 and 10 mM. The inset shows experimental data published by Shimoni et al.[16] obtained at different $[K^+]_o$ levels. Note that the original ORd model for I_{K1} does *not* reproduce this well-established pattern of changes in I_{K1}, in response to these changes in $[K^+]_o$ whereas GPB formulation has this essential capability. See Results and Discussion for further explanation.

Supplementary Materials

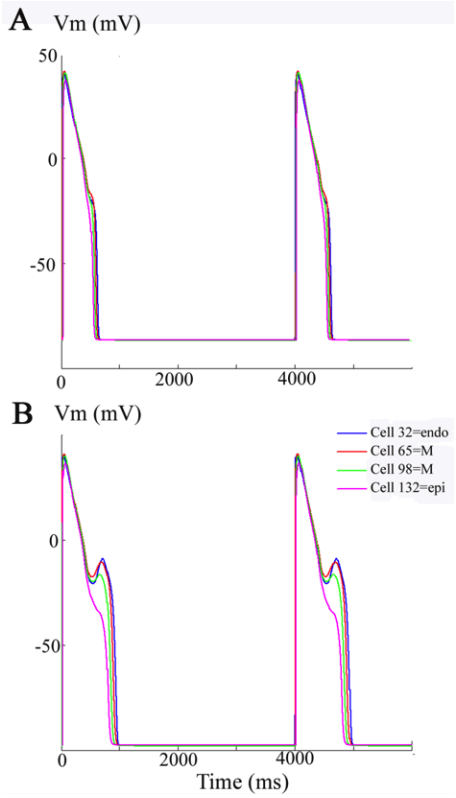

Figure S-3. Evaluation of the effects of low $[K^+]_o$ on EAD propagation based on computations done using our modified ORd model of the human ventricular action potential (AP). The chosen *in silico* ventricular myocyte 'substrate' corresponds to a transmural 1-D strand or cable: 165 myocytes were arranged end-to-end in a strand. A suprathreshold stimulus applied to the extreme endocardial end of this strand elicited an AP that propagated through the endocardial, M, and epicardial myocytes. The patterns of responses in $[K^+]_o$ 5.4 and 3.5 mM are represented in Panels A and B, respectively. To enhance the likelihood that early after-depolarizations (EADs), a 70% block of the rapid delayed rectifier current (I_{Kr}) and a 20% enhancement of the late sodium current (I_{NaL}) were introduced. The stimulus basic cycle length (BCL) was 4000 ms and intracellular conductivity was set to achieve a conduction velocity of 41 cm/s.[93,94] The

colors of the APs correspond to the selected regions (cell number) within the strand: cell 32 – endo (blue); cell 65 – M (red); cell 98 – M (green); cell 132 – epi (magenta). See text for further explanation. ‘M’ denotes mid-myocardium.