Does the combination of hyperkalemia and $K_{ATP}$ activation determine excitation rate gradient and electrical failure in the globally ischemic fibrillating heart?

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Taylor TG, Venable PW, Booth A, Garg V, Shibayama J, Zaitsev AV. Does the combination of hyperkalemia and $K_{ATP}$ activation determine excitation rate gradient and electrical failure in the globally ischemic fibrillating heart? Am J Physiol Heart Circ Physiol 305:H903–H912, 2013. First published July 19, 2013; doi:10.1152/ajpheart.00184.2013.—Ventricular fibrillation (VF) in the globally ischemic heart is characterized by a progressive electrical depression manifested as a decline in the VF excitation rate (VFR) and loss of excitability, which occur first in the subepicardium (Epi) and spread to the subendocardium (Endo). Early electrical failure is detrimental to successful defibrillation and resuscitation during cardiac arrest. Hyperkalemia and/or the activation of ATP-sensitive $K^+$ ($K_{ATP}$) channels have been implicated in electrical failure, but the role of these factors in ischemic VF is poorly understood. We determined the VFR-extracellular K\(^{\text{o}}\) concentration ([K\(^{\text{o}}\)]) relationship in the Epi and Epi of the left ventricle during VF in globally ischemic hearts (Isch group) and normoxic hearts subjected to hyperkalemia (HighK group) or a combination of hyperkalemia and the $K_{ATP}$ channel opener cromakalim (HighK-Crom group). In the Isch group, Endo and Epi values of [K\(^{\text{o}}\)] were compared in the early (0–6 min), middle (7–13 min), and late (14–20 min) phases of ischemic VF. A significant transmural gradient in VFR (Endo > Epi) was observed in all three phases, whereas a significant transmural gradient in [K\(^{\text{o}}\)] (Epi > Endo) occurred only in the late phase of ischemic VF. In the Isch group, the VFR decrease and inexcitability started to occur at much lower [K\(^{\text{o}}\)] than in the HighK group, especially in the Epi. Combining $K_{ATP}$ activation with hyperkalemia only shifted the VFR-[K\(^{\text{o}}\)] curve upward (an effect opposite to real ischemia) without changing the [K\(^{\text{o}}\)] threshold for asystole. We conclude that hyperkalemia and/or $K_{ATP}$ activation cannot adequately explain the heterogeneous electrical depression and electrical failure during ischemic VF.

extracellular potassium accumulation; myocardial ischemia; ventricular fibrillation; ATP-sensitive potassium channel; asystole

HETEROGENEOUS ELECTRICAL DEPRESSION and the eventual loss of excitability occurring in the course of cardiac arrest are major adverse outcomes determining the incidence of reperfusion arrhythmias (1) and asystole, with the latter usually being the point of ultimate failure in resuscitation practice (10). In the ex vivo blood-perfused canine heart, simulated cardiac arrest produced by a combination of ventricular fibrillation (VF) and global ischemia causes a prominent transmural gradient in the VF rate (VFR) followed by the emergence of inexcitable areas in the epicardium (Epi), which spread across the ventricular wall and culminate in global asystole within a clinically relevant timeframe (32, 34). The transmural VFR gradient is also present in explanted human hearts subjected to global ischemia and VF (9). Given the similarity between the canine heart and human heart, understanding the mechanisms of heterogeneous electrical depression in the canine model may help to improve the outcomes of resuscitation from cardiac arrest in patients, based on mechanistic knowledge.

Electrical depression during ischemia without VF has been studied for many years, and the classical teaching posits that the main factor determining excitability during ischemia is the elevation of the extracellular K\(^{+}\) concentration ([K\(^{+}\)]\(_{\text{o}}\); hyperkalemia), which leads to depolarization of the resting potential and a subsequent reduction in the availability of Na\(^{+}\) channels for activation (30). Moreover, because Na\(^{+}\) channel inactivation occurs at more negative potentials in the Epi than in the endocardium (Endo), even a transmurally uniform increase in [K\(^{+}\)]\(_{\text{o}}\) can bring about a selective depression of Epi action potentials and transmural frequency-dependent conduction block (6). Thus, assuming that the primary sources of high-frequency activation during ischemic VF are harbored by Endo Purkinje fibers (8), global uniform hyperkalemia may be sufficient to explain the VFR gradient during ischemic VF. However, this possibility has not been tested in experiments. The second factor implicated in the loss of excitability during ischemia is activation of ATP-sensitive K\(^{+}\) ($K_{ATP}$) channels, which, in theory, can oppose sarcolemmal depolarization caused by the opening of Na\(^{+}\) channels to the degree at which spread of excitation becomes impossible, leading to so-called “sink block” (1). Moreover, the two factors (hyperkalemia and $K_{ATP}$ activation) in theory can be synergistic, such that in the presence of $K_{ATP}$ activation, conduction block would occur at lower [K\(^{+}\)]\(_{\text{o}}\) than hyperkalemia alone (30). To the best of our knowledge, however, such synergism has not been tested in experimental studies.

Cardiac arrest secondary to VF presents a more complex context than ischemia alone because the high excitation rate of VF aggravates metabolic challenge by increasing energy demand (16) and can potentially contribute to ionic imbalances that develop in the course of ischemia. For example, it was shown that the high excitation rate present during atrial fibrillation causes a moderate but detectable elevation of [K\(^{+}\)]\(_{\text{o}}\), even in the presence of coronary circulation (19). It is thus possible that VF in globally ischemic hearts would aggravate hyperkalemia, but the time course of [K\(^{+}\)]\(_{\text{o}}\) elevation in ischemic VF has not yet been reported.

The relationship between ionic alterations caused by ischemia and the excitation rate of VF is complex and remains controversial. The VF cycle length, the inverse of VFR, is correlated with the duration of the refractory period, which, in turn, is determined by the sum of the action potential duration and the duration of the period after repolarization during which
were exposed via a midline sternotomy and rapidly excised for perfusion on a Langendorff apparatus with a mixture of heparinized blood and Tyrode solution as previously described (12, 37). After isolation, hearts were placed in either a vapor chamber or immersed in Tyrode superfusion to maintain an Epi temperature of 37 ± 1°C. Twenty-six hearts were subjected to 20 min of ischemic VF by inducing VF with a 9-V battery 10 s before the onset of global ischemia initiated by the interruption of aortic perfusion (Isch group). In these hearts, [K+]e increased naturally during ischemia and was measured using K+-sensitive electrodes (see below). In the remaining 10 hearts, VF was induced in the same way, and normal oxygenated perfusion was maintained in the fibrillating heart while [K+]e was varied between 4 and 15 mM by altering [K+]p in the perfusate. In five of these hearts, hyperkalemia was the only factor tested (HighK group). In the remaining five hearts, 20–34 μM cromakalim (a KATP channel opener) was administered before [K+]o was increased (HighK-Crom group). In the HighK and HighK-Crom groups, [K+]e was increased stepwise by adding 1–3 ml of 600 mM KCl solution at a time to the recirculating blood-Tyrode mixture while the resulting [K+]p in the perfusate was measured every 2–3 min using a NOVA 8 ion analyzer (Nova Biomedical, Waltham, MA). A VF measurement was accepted for analysis if it was bracketed by two venous [K+]o measurements within 0.5 mM of each other, and the average of the two [K+]o values was used for analysis.

K+-sensitive electrodes and [K+]o measurements. K+-sensitive electrodes were prepared following as close as possible the process described by Johnson et al. (13). However, the electrodes manufactured in this fashion turned out to be extremely fragile, with many not surviving the experimental protocol. To improve our success rate, the prepared electrodes were then housed in a 20-gauge hypodermic needle with an apature exposing the sensing part of the K+-sensitive electrode to the interstitial fluid (Fig. 1, A and B). The depth of the K+-sensitive electrode could be adjusted by sliding the thin polymer tubing (arrow in Fig. 1A) along the shaft of the needle. The assembled K+-sensitive electrode and housing are referred to as “K+ needle” below.

K+ needles were calibrated both before and after the experiment using 3 and 10 mM KCl solution; any electrode that showed a voltage shift outside the range of 30 ± 4 mV before or after the experiment was excluded as damaged, and the data collected was discarded. Figure 1C shows an example calibration of Endo and Epi K+ needles. Note that both electrodes showed stable measurements over 30-min periods in both 3 and 10 mM KCl solution with an ~30-mV shift between solutions. The inset in Fig. 1C shows the rapid (in the order of seconds) response of the K+ needles to the transition from 3 to 10 mM solution. Millivolt values were converted to millimolar values using the Nernst equation [for details of the calibration, see Johnson et al. (13)]. Since the calibration constant could be slightly different pre- and postexperiment, we used the average of the pre- and post-experimental calibration values for conversion purposes. In addition, the response of K+ needles placed in hearts was tested by adding a bolus of 600 mM KCl solution to the recirculating blood-Tyrode mixture. Figure 1D shows an example of recordings from Endo and Epi K+ needles (squares and triangles, respectively) verified by measurements using a blood ion analyzer (large Xs). The initial value of [K+]o in the perfusate was 2.2 mM. The indicated target concentration was calculated based on the estimated dilution factor. The two vertical dashed lines indicate the timing of bolus injection and washout. Washout was performed by replacing ~700 ml of the K+-enriched perfusate with an equivalent amount of a surplus blood-Tyrode mixture. Since all of the blood with elevated [K+]o could not be removed (due to the limited volume of the blood-Tyrode mixture), the final [K+]o remained elevated (5.4 mM) compared with before the K+ bolus (2.2 mM). Note, however, that during steady-state conditions both before and after the K+ bolus there was a close match between [K+]o values obtained by K+ needles and the blood ion analyzer.

METHODS

This investigation conformed with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Pub. No. 85-23, Revised 1996) and was approved by the Institutional Animal Care and Use Committee of the University of Utah (Protocol No. 10–00005).

Experimental protocol. A total of 36 dogs (12 mongrel dogs and 24 purpose-bred dogs, weight: 24.6 ± 0.7 kg, 20 male and 16 female) were euthanized for this study. Animals were premedicated with acepromazine (0.4 mg/kg), propropofol (8 mg/kg), or Telazol (9 mg/kg), and a deep plane of anesthesia was maintained with pentobarbital in all dogs (initial dose: 50 mg/kg, additional doses as needed to maintain deep anesthesia for the duration of surgery, <1 h). Hearts
needles were placed in the anterior left ventricular (LV) free wall. Epi needles were manufactured to have the sensor reliably submerged into the tissue, and the actual depth of the Epi measurement was 3 mm from the epicardial surface. The depth of the Endo measurement could not be determined at the moment of the needle insertion due to the trabeculated structure of the LV Endo (in our estimates, the thickness of the LV wall varied between 5 mm at the thinnest part and 20 mm at locations where the papillary muscles were attached to the LV free wall). Thus, it was not possible to have a fixed depth of the sensing aperture in Endo needles. To address the irregular thickness of the LV free wall and to make sure that the sensing portion of the K⁺ needle was located close to the Endo surface, we used two complementary approaches. First, we performed "pacing threshold mapping" using one of the multicontact plunge needle electrodes used for recording unipolar electrogams across the LV wall (see below). In essence, we applied pacing stimuli to consecutive pairs of adjacent leads (starting from the most Endo leads: 1 and 2, 2 and 3, etc.) and measured the end-diastolic threshold for excitation. Based on the observation that Endo pacing thresholds were typically the lowest across the LV wall and very high in the LV cavity (not shown), the pacing cathode location yielding the lowest excitation threshold between 5 and 16 mm from the Epi surface was

![Fig. 1. Custom-made K⁺-sensitive electrodes. A: K⁺-sensitive electrodes were enclosed in a 20-gauge hypodermic needle that contained a window (white box) that exposed the K⁺ sensor to the myocardial tissue. The polymer tubing (arrow) could be moved along the shaft of the needle to adjust the position of the K⁺ sensor at the desired distance from the epicardial (Epi) surface. B: close up of the sensing part of the K⁺ needle indicated by the white box in A. This design provided both adjustable depth and protection for the delicate sensor. C: example of calibration of endocardial (Endo) and Epi K⁺ needles. Note that both electrodes showed stable measurements over 30-min periods in both 3 and 10 mM KCl solution with an ~30-mV shift between solutions. The inset shows the rapid (in the order of seconds) response of the K⁺ needles to the transition from 3 to 10 mM solution. D: response of Endo (squares) and Epi (triangles) K⁺ needles placed in a heart to a bolus of 600 mM KCl solution added to the recirculating blood-Tyrode mixture. The indicated target concentration was calculated based on the estimated dilution factor. The two vertical dashed lines indicate the timing of bolus injection and (partial) washout. Note that during steady-state conditions both before and after the KCl bolus, there was a close match between extracellular K⁺ concentration ([K⁺]o) values obtained by K⁺ needles and those obtained using the blood ion analyzer (large Xs).](http://ajpheart.physiology.org/)

K⁺ needles were placed in the anterior left ventricular (LV) free wall. Epi K⁺ needles were manufactured to have the sensor reliably submerged into the tissue, and the actual depth of the Epi [K⁺]o measurement was ~3 mm from the epicardial surface. The depth of the Endo [K⁺]o measurement could not be determined at the moment of the needle insertion due to the trabeculated structure of the LV Endo (in our estimates, the thickness of the LV wall varied between 5 mm at the thinnest part and 20 mm at locations where the papillary muscles were attached to the LV free wall). Thus, it was not possible to have a fixed depth of the sensing aperture in Endo K⁺ needles. To address the irregular thickness of the LV free wall and to make sure that the sensing portion of the K⁺ needle was located close to the Endo surface, we used two complementary approaches. First, we performed "pacing threshold mapping" using one of the multicontact plunge needle electrodes used for recording unipolar electrograms across the LV wall (see below). In essence, we applied pacing stimuli to consecutive pairs of adjacent leads (starting from the most Endo leads: 1 and 2, 2 and 3, etc.) and measured the end-diastolic threshold for excitation. Based on the observation that Endo pacing thresholds were typically the lowest across the LV wall and very high in the LV cavity (not shown), the pacing cathode location yielding the lowest excitation threshold between 5 and 16 mm from the Epi surface was
considered to be the best estimate for the Endo location. With this estimate, the depth of the sensing window on the Endo K⁺ needle was adjusted by sliding the Epi cuff (see arrow in Fig. 1A) along the shaft of the needle, and the needle was inserted within 2 mm of the multicontact electrode used for the pacing mapping. Second, to obtain the actual position of sensors in Endo K⁺ needles, we exposed the LV Endo after finishing our experimental protocol and visually determined the distance of the sensing portion of the K⁺ needles from the Endo. The accepted Endo [K⁺]₀ measurements were collected at a distance of 1–6 mm from the Endo surface. One to three Epi K⁺ needles and one to three Endo K⁺ needles were used in each experiment, with the goal of having at least one measurement from both the Endo and Epi after discardng any electrodes not meeting our requirements for calibration and the intramural position of the sensor. In those experiments in which two or (rarely) three acceptable K⁺ recordings were obtained either from the Epi or Endo, the data from these individual measurements were averaged to represent the respective layer in that particular experiment.

Electrical recordings and analysis. Plunge needle electrodes with 10 evenly spaced unipolar leads (interlead distance: 1.6 mm) were manufactured in house following the design developed by Rogers et al. (27). Ten needles were placed across the anterior surface of the heart, and three needles in close proximity to the K⁺ needles were chosen for analysis. The distance between needles was 10–15 mm. Unipolar electrograms from all contacts of the needle electrodes were recorded continuously during the first 20 min of ischemic VF at a sampling rate of 1 kHz using a custom-made multichannel data-acquisition system (31). Unipolar electrograms were analyzed using custom software developed in the Matlab (Mathworks, Natick, MA) framework. Electrograms were first filtered using a 60-Hz notch filter including several shorter harmonics. We applied the Hilbert transform to unipolar electrograms as previously described (18, 23, 32). Briefly, the Hilbert transform converts a fluctuating voltage signal into its corresponding phase so that consecutive cycles of activation are given an estimate of frequency. The number of phase transitions from 2π to −2π gives an estimate of frequency. To decrease the influence of noise, activation cycles with a length of <33 ms and amplitude of the waveform between two consecutive phase transitions of <10% of the electrogram amplitude measured at 0 min of ischemic VF were excluded from the total cycle counts. After that, VFR was calculated as the average number of activations per second over 4-s intervals taken at 4 s after ischemic VF induction and at minutes 1–20 of ischemic VF. For simplicity, the first time point is referred to as 0 min of ischemic VF. Local tissue was considered inexitable if VFR was 0 Hz (no detectable activations). The percentage of Endo or Epi leads that were inexitable was determined at each time point of ischemia.

Whereas the length of the plunge needle electrodes was constant and designed to span the largest thickness observed in the LV of the canine heart, as previously mentioned the actual thickness of the ventricular wall is highly nonuniform. Thus, in some cases, plunge needle electrodes were longer than the wall thickness at the site of insertion. In the beginning of this study, we used our elaborate set of criteria to exclude the contacts protruding from the ventricular wall based on the analysis of the electrograms that we previously described (34). However, in the vast majority of experiments, we determined the relationship between plunge needle length and LV wall thickness by visual inspection and wall thickness measurements after opening the LV cavity at the end of the experiments, simultaneous with determining the depth of sensor location in Endo K⁺ needles.

Plots of VFR and percentage of inexitable sites versus [K⁺]₀. VFR and the percentage of inexitable sites in the Endo and Epi were plotted against measured [K⁺]₀ for all three experimental groups (Isch, HighK, and HighK-Crom groups). Data were grouped into increments of 1 mM [K⁺]₀, for comparison. In some experiments from the HighK and HighK-Crom groups, [K⁺]₀ measurements were sparse and were apart by >1 mM. In these cases, VFR and the percentage of inexitable sites for intermediate [K⁺]₀ values were linearly interpolated. In addition, in some early experiments from the HighK and HighK-Crom groups when full asystole was observed at [K⁺]₀ between 10 and 15 mM, higher levels of [K⁺]₀ were not tested. In those cases, we assumed that VFR would remain at 0 Hz and that the percentage of inexitable sites would remain at 100%, for all [K⁺]₀ above the highest [K⁺]₀, for which asystole was actually observed.

Statistical analysis. All curves were compared using two-way ANOVA. The time course of VFR and [K⁺]₀ during ischemic VF was compared separately for three phases: early (0–6 min), middle (7–13 min), and late (14–20 min) ischemia. Data are given as means ± SE. Differences of P < 0.05 were considered statistically significant.

RESULTS

Time course of [K⁺]₀, VFR, and the occurrence of inexcitability during ischemic VF. Figure 2A shows the time course of [K⁺]₀ elevation in the Endo and Epi during ischemic VF. Extracellular K⁺ accumulation started immediately upon the onset of ischemic VF and monotonically increased over the entire 20-min ischemic interval. The total increase (∆[K⁺]₀) was greater in the Epi than in the Endo (8.3 vs. 6.6 mM). However, the Epi-to-Endo difference was statistically significant only during the late phase of ischemic VF (14–20 min). This relationship persisted when the Endo [K⁺]₀ measurements for analysis were limited only to those collected within 0–3 mm from the Endo surface (not shown). The transmural difference in VFR (Fig. 2B) was much more pronounced than the difference in [K⁺]₀, and was statistically significant throughout all three phases of ischemic VF, consistent with previous studies (8, 34, 36). When measurements with VFR = 0 were excluded from analysis, the transmural VFR difference was overall similar and remained statistically significant throughout all three phases of ischemic VF (not shown). Figure 2C shows the percentage of electrodes with undetectable electrical activity (thus deemed inexitable according to the formal criterion; see METHODS) as a function of ischemia duration. As shown in Fig. 2C, the prevalence of inexitable sites steadily increased during ischemic VF in both the Endo and Epi, but at a much higher rate in the Epi than in the Endo. The transmural difference in this parameter was statistically significant during all three phases of ischemic VF. Note that the largest Endo-to-Epi difference in VFR and excitability was observed during the middle phase of ischemic VF (7–13 min), after which time point these parameters converged, indicating nearly complete electrical suppression by 20 min of ischemia.

Relationship between [K⁺]₀ and VFR under various experimental conditions. To assess the role of hyperkalemia in the progressive VF decline and eventual inexcitability during ischemic VF, we compared the relationship between VFR and [K⁺]₀ during ischemic VF (Isch group), normoxic VF in the presence of hyperkalemia (HighK group), and normoxic VF in the presence of hyperkalemia in combination with the KᵦTP channel opener cromakalim (HighK-Crom group). In normoxic experiments, [K⁺]₀ was varied between 4 and 15 mM. Note that in ischemic hearts, the elevated K⁺ concentration was achieved naturally, whereas in all other experiments it was adjusted by adding boluses of KCl to the perfusate (see METHODS).

Figure 3 shows examples of transmural unipolar electrograms recorded at approximately normal [K⁺]₀ (3.7–4.3 mM;
left) and at high $[K^+]_o$ (9.1–9.4 mM; right) in the three experimental groups (from top to bottom: Isch, HighK, and HighK-Crom groups). Figure 3, top left, shows electrograms obtained at 0 min of ischemic VF when the level of $[K^+]_o$ was 3.7 mM. Figure 3, top right, shows electrograms obtained at 7 min of ischemic VF when the level of $[K^+]_o$ was 9.1 mM in the Epi and 9.3 mM in the Endo. As was the typical situation at 0 min and between 5 and 9 min of ischemic VF (see Fig. 2A), in this example, there was no significant difference between $[K^+]_o$ in the Epi and Endo. As shown in Fig. 3, at 0 min of ischemia and $[K^+]_o = 3.7$ mM, there was no perceptible difference in VFR between the Epi and Endo. In contrast, at 7 min of ischemia and $[K^+]_o \sim 9$ mM, there was a drastic difference in VFR between the Epi and Endo, despite the relative uniformity of $[K^+]_o$. In fact, the outer half of the LV wall did not have detectable activations at all (VFR = 0).

Figure 3, middle, shows a representative example of transmural unipolar electrograms from a HighK heart subjected to $[K^+]_o = 3.7$ mM (left) and $[K^+]_o = 9.3$ mM (right). As shown in Fig. 3, middle, at both levels of $[K^+]_o$, VFR was uniform across the ventricular wall and the actual VFR was virtually unaffected by the increase in $[K^+]_o$ from 3.7 to 9.3 mM. Figure 3, bottom, shows a representative example of transmural unipolar electrograms from a HighK-Crom heart subjected to $[K^+]_o = 4.3$ mM (left) and $[K^+]_o = 9.4$ mM (right). As shown in Fig. 3, bottom, activation of $K_{ATP}$ channels by cromakalim induced a huge and transmurally uniform increase in VFR compared with the absence of the drug. Note that VFR remained exceedingly high even when $[K^+]_o$ was raised to 9.4 mM, with no appreciable transmural VFR gradient. Moreover, note the striking contrast between VFR during real ischemia (Fig. 3, top right) and VFR during hyperkalemia in combination with $K_{ATP}$ activation (Fig. 3, bottom right), despite the same level of $[K^+]_o$.

Figure 4A shows the statistical summary of the VFR-$[K^+]_o$ relationship in all three experimental groups. The different colors represent the different experimental groups; open triangles and solid squares represent Epi VFR and Endo VFR, respectively. The diagram shown in the inset in Fig. 4A shows relevant pairwise comparisons. The vertical brackets indicate statistically significant difference between the same layer in different experimental groups; the horizontal brackets indicate statistically significant difference between the Endo and Epi in the same experimental group. As shown in Fig. 4, in the HighK group (red), an appreciable decrease in VFR in both the Endo and Epi occurred only at $[K^+]_o$ exceeding 8 mM and VFR approached zero at $[K^+]_o$ between 12 and 14 mM. Even though

Fig. 2. Time course of $[K^+]_o$ (A), ventricular fibrillation (VF) rate (VFR; B), and the percentage of inexcitable sites (%Inexcitible; C) during 20 min of ischemic VF. The open triangles and filled squares represent the Epi and Endo, respectively. The vertical dashed lines denote the early, middle, and late phases of ischemic VF. A: $[K^+]_o$, progressively increased in both the Epi and Endo throughout 20 min of ischemic VF. Note that the difference between Endo and Epi $[K^+]_o$ was statistically significant ($P < 0.05$) only during the late phase of ischemic VF. B: time course of VFR decline. VFR decreased faster in the Epi than in the Endo, creating a prominent and statistically significant Endo-to-Epi difference during all phases of ischemic VF. The magnitude of the VFR gradient eventually decreased, as both Epi and Endo VFR values converged to zero by 20 min of ischemic VF. C: progressive increase in the percentage of inexcitable sites. The Epi showed a more rapid onset and greater extent of local inexcitability compared with the Endo, but both Endo and Epi locations approached 100% inexcitability by the end of 20-min episodes of ischemic VF. The Endo-to-Epi difference in the percentage of inexcitable sites was statistically significant during all phases of ischemic VF. Note the discrepancy between the dynamics of the $[K^+]_o$ gradient and the gradients of VFR and excitability: the largest transmural difference in the electrical parameters was observed when there was no transmural difference in $[K^+]_o$, and vice versa. *$P < 0.05$; **$P < 0.0001$. 

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Endo VFR appeared to be slightly higher than Epi VFR at each tested $[K^+]_o$, the difference was not statistically significant. In the Isch group (black in Fig. 4), the relationship between $[K^+]_o$ and VFR was profoundly different from that in the HighK group. First, at each $[K^+]_o$, VFR in the Isch group was lower than the respective VFR in the HighK group. Second, in the Isch group, there was also a large and significant difference in the sensitivity of VFR to $[K^+]_o$ between the Epi and Endo. Note that the average Epi VFR was much lower than the Endo VFR in the range of $[K^+]_o$ between 6 and 9 mM. Also, uniquely among all groups, the average Epi VFR in the Isch group was very slow (<1 Hz) starting from a concentration of $[K^+]_o$ equal to 8 mM. This made a stark contrast with the HighK group, in which at 8 mM $[K^+]_o$, Epi VFR was barely different from that observed at normal $[K^+]_o$.

The combination of hyperkalemia and $K_{ATP}$ activation by cromakalim (HighK-Crom group; blue in Fig. 4) poorly reproduced the VFR-$[K^+]_o$ relationship observed during ischemia. $K_{ATP}$ activation caused a massive increase in VFR, which was maintained at all $[K^+]_o$ between 4 and 11 mM, in contrast to VFR slowing observed in the Isch group. Even though in the HighK-Crom group there was a trend of slower VFR in the Epi than in the Endo, especially at $[K^+]_o$ between 7 and 8 mM, the transmural VFR difference was not statistically significant.

Figure 4B shows the statistical summary of the relationship between $[K^+]_o$ and the percentage of inexcitable LV locations.
Note that in the Epi, almost 50% of recorded locations were inexcitable at \( [K^+]_o \), as low as 6 mM. In contrast, both HighK and HighK-Crom groups showed a significant loss of excitability only at \( [K^+]_o \), above 12 mM, thus approaching the canonical level of \( K^+ \)-induced inexcitability (30). It is of interest that \( K_{ATP} \) activation did not enhance the loss of excitability in the presence of hyperkalemia, despite the theoretical possibility of a synergistic relationship between these two factors with respect to electrical depression (30).

**DISCUSSION**

VF is a frequent cause of sudden cardiac arrest and, in combination with ensuing global myocardial ischemia, determines the context of cardiopulmonary resuscitation and other life-saving procedures. VF evolving in globally ischemic hearts (sometimes termed long-duration VF) is a highly dynamic process featuring progressive transmural dispersion of VFR (8, 34, 36), which, at least in part, reflects the transmural heterogeneous increase in postrepolarization refractoriness (15, 34). This increase is unbounded and culminates in a complete loss of excitability first in the LV Epi and subsequently in the rest of the ventricles (32, 34). Total loss of excitability (asystole) is in many cases the early point of no return after out-of-hospital cardiac arrest. Regional inexcitability can contribute to postperfusion lethal arrhythmias (1), which is a major complication of cardiac arrest in the aftermath of successful defibrillation (33).

Despite the obvious significance of progressive electrical depression in the course of ischemic VF, little is known about the major determinants of this phenomenon. Shaw and Rudy (30) used a numeric model to analyze the relative roles of hyperkalemia, acidosis, and \( K_{ATP} \) activation in conduction failure during ischemia. In this study, elevation of \( [K^+]_o \), to \( \sim 14 \text{ mM} \) was fully sufficient to cause conduction block. Acidosis did not have a significant additional effect, but a large degree of \( K_{ATP} \) activation (20% channel availability assuming an intracellular ATP concentration of 0.5 mM) lowered the threshold \( [K^+]_o \) necessary to cause conduction block from 14 to 10 mM. From this, it would be reasonable to assume that elevated \( [K^+]_o \) and a massive activation of \( K_{ATP} \) channels are major determinants of electrical failure during ischemic VF.

**Hyperkalemia.** To the best of our knowledge, our study is the first to measure the progressive accumulation of \( [K^+]_o \) during ischemic VF. We found a larger \( K^+ \) efflux in the Epi than in the Endo, but the difference was significant only in the late phase of ischemic VF (14-20 min). We can compare these results only to previous studies performed in nonfibrillating hearts. In an in situ canine model of regional ischemia (20, 21), there was no transmural difference in \( [K^+]_o \), and the total \( K^+ \) accumulation in the Epi and Endo during 20 min of left anterior descending coronary artery (LAD) occlusion was similar to that observed in the Endo after 20 min of ischemic VF in our study (\( \sim 9-10 \text{ mM} \)). In another study (7) using isolated blood-perfused canine hearts, \( [K^+]_o \) was measured in the regionally ischemic myocardium at a depth of 5 mm from the Epi surface during pacing at a normal heart rate. In that study, at the end of an 8-min LAD occlusion period, the level of \( [K^+]_o \) in the ischemic zone reached \( \sim 8 \text{ mM} \), which was very close to both Endo and Epi values of \( [K^+]_o \) observed at the same duration of ischemia in our study (see Fig. 2B).

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**Fig. 4.** Statistical analysis of VFR (A) and the percentage of inexcitable sites (B) as functions of \( [K^+]_o \), in the Isch, HighK, and HighK-Crom groups (black, red, and blue, respectively). In each group, the Epi and Endo are represented by open triangles and filled squares, respectively. The insets in both A and B indicate relevant pairwise comparisons that yielded statistically significant difference (\( P < 0.05 \)). The vertical brackets indicate differences between experimental groups in a given myocardial layer; the horizontal brackets indicate differences between the Endo and Epi in the same experimental group. A: VFR as the function of \( [K^+]_o \). VFR in the Isch group was lower, whereas VFR in the HighK-Crom was higher, than VFR in the HighK group at all \( [K^+]_o \), between 4 and 11 mM. Transmural differences in VFR were statistically significant only in the Isch group. Note that hyperkalemia combined with \( K_{ATP} \) activation did not reproduce the \( [K^+]_o \)-VFR relationship observed during ischemia. B: percentage of inexcitable sites as a function of \( [K^+]_o \). Note the difference between the Isch group and the two normoxic groups (HighK and HighK-Crom groups). In the Isch group, inexcitable sites appeared at relatively low \( [K^+]_o \), and there was a large difference in the percentage of inexcitable sites between the Epi and Endo at intermediate levels of \( [K^+]_o \). In contrast, both the HighK and HighK-Crom groups started to exhibit a loss of excitability only at \( [K^+]_o \), above 10 mM, and there was no transmural difference in the percentage of inexcitable sites. Note that the upper limit of excitability in all experimental groups and myocardial layers was at \( [K^+]_o \) \( \sim 14-15 \text{ mM} \), a level consistent with the inactivation of fast \( Na^+ \) channels, which are necessary to maintain propagation of cardiac impulse (30).

\( \text{VFR} = 0 \) in all tested groups. Note that this information is somewhat related to the relationship between \( [K^+]_o \) and VFR shown in Fig. 4A, especially given the fact that sites with VFR = 0 were included in the average VFR values shown in Fig. 4A. Yet, the results shown in Fig. 4B underscore the uniqueness of the ischemic data, especially in the Epi, in that during ischemic VF loss of excitability occurred at very modest levels of \( [K^+]_o \).
sured the incidence of inexcitability as the percentage of unipolar electrograms exhibiting “monophasic” morphology and found that after 8 min of ischemia, only ~25% of recording sites were inexcitable (see Fig. 5A in Ref. 7). In our case, the degree of inexcitability was drastically different between the Endo and Epi. Whereas in the Endo the percentage of inexcitable sites at 8 min of ischemia (28%) was similar to that observed by Coronel et al. (7) in the midmyocardium, in the Epi at the same time point of ischemia, it was much larger (63%). In stark contrast to ischemic conditions, raising [K+]o to the level of 8 mM during normal perfusion in our study never caused inexcitability in any layer of the LV wall, and even the effect with respect to VFR was minimal (see Fig. 4A). These results disagree with those obtained during ischemic VF in the isolated rabbit heart, where an elevation of [K+]o to 8 mM in normoxic hearts reproduced the decrease in VFR observed during ischemia (4). Apart from the differences in species, another important difference between the rabbit study and our study is the degree of ischemia, which was complete (no flow) in our experiments and was only partial (remaining flow at 15% of normal) in the study by Caldwell et al. (4).

Because Na+ channel inactivation occurs at more negative potentials in the Epi than in the Endo, even a transmurally uniform increase in [K+]o can cause differential electrical depression in the Epi and Endo, leading, in particular, to the inability of the Epi to follow a rapid rate of excitation waves coming from the Endo (6). If this effect were significant in our study, we would expect that uniform hyperkalemia due to an elevation of [K+]o, in the perfusate would induce the transmural difference in VFR and/or the percentage of inexcitable sites. In fact, neither of these occurred in our study. Hyperkalemia in normoxic hearts caused a transmurally uniform VFR decline at [K+]o above 8 mM, culminating in a transmurally uniform loss of excitability when [K+]o exceeded 12–14 mM. Thus, it is unlikely that the differential properties of Na+ channel inactivation (6) contribute significantly to the transmural differences in VFR and excitability observed during ischemic VF.

K<sub>ATP</sub> channels. The timing, conditions, and electrophysiological consequences of K<sub>ATP</sub> activation during real ischemia in intact hearts remain as controversial now as three decades ago, when the channel was first discovered (25). While the principal role of K<sub>ATP</sub> channels in ischemic action potential duration shortening appears to be undisputable, its potential contribution to decreased membrane excitability and conduction failure during ischemia is far less clear. In their theoretical analysis of factors influencing conduction failure during early ischemia, Shaw and Rudy (30) concluded that the contribution of K<sub>ATP</sub> activation to conduction failure is limited unless the degree of activation of this channel is very large, which would require intracellular ATP concentration to reach very low (submillimolar) levels, much below what is typically observed within the first 10–15 min of ischemia. However, since it is not possible to directly measure activation of K<sub>ATP</sub> channels in intact tissues during ischemia, and because the regulation of this channel is dependent on a number of metabolites besides ATP, the possibility of any degree of K<sub>ATP</sub> activation during early ischemia remains open. Akar et al. (1) asserted the role of K<sub>ATP</sub> channel opening as the primary mechanism of ischemic inexcitability by an experimental demonstration of “metabolic sink block” resulting from K<sub>ATP</sub> activation secondary to mitochondrial depolarization (1). Noteworthy, in isolated ventricular cardiomyocytes, complete loss of excitability can occur without any increase in [K+]o, provided that the K<sub>ATP</sub> channel is sufficiently activated in response to mitochondrial depolarization (1, 22).

Since hyperkalemia alone failed to reproduce the [K+]o-VFR relationship observed during ischemic VF, we tested the possibility that in the presence of a strong activation of K<sub>ATP</sub> channels, the [K+]o-VFR relationship observed during normoxic VF would approach more closely the relationship observed during ischemic VF. However, this was not the case. The K<sub>ATP</sub> opener cromakalim caused a dramatic increase in VFR in the range of [K+]o between 5 and 10 mM, which was opposite to the effect of ischemia. Also, cromakalim did not decrease the critical [K+]o at which excitability is lost (it remained in the range of 12–14 mM). The concentration of cromakalim used in this study is expected to activate ~50% of the channels (24), which exceeds even what Shaw and Rudy (30) considered to be a result of extreme anoxia (20% of channels available). Yet even such an extreme level of K<sub>ATP</sub> activation failed to cause VFR suppression or loss of excitability up to [K+]o of 12 mM, the concentration at which ~40% of the Endo locations and ~80% of the Epi locations exhibited loss of activity during ischemic VF (see Fig. 4B). These observations speak against the principal role of K<sub>ATP</sub> activation in electrical depression during ischemic VF, somewhat contradicting the concept of metabolic sink block (1) and our own previous finding that the blockade of K<sub>ATP</sub> channels by glybenclamide slightly postponed VFR decline and the emergence of local and global inexcitability during ischemic VF (32). To reconcile these apparent discrepancies, we suggest that K<sub>ATP</sub> activation does contribute to electrical depression during ischemic VF, but perhaps only in the presence of other ischemic factors yet to be established.

Possible additional factors. The Na<sup>+</sup>-activated K<sup>+</sup> (K<sub>Na</sub>) channel could theoretically contribute to electrical depression in the course of ischemic VF. This channel has conductance even larger than that of K<sub>ATP</sub> channels (35), and, thus, the consequences of its activation during ischemia could be similar to those of K<sub>ATP</sub> activation [i.e., sink block (1)]. The effects of K<sub>ATP</sub> and K<sub>Na</sub> activation could be synergistic with respect to electrical depression. Even though the available experimental evidence speaks against functional expression of K<sub>Na</sub> channels in the sarcolemma of canine ventricular myocytes (28), one cannot exclude that the lack of functioning K<sub>Na</sub> channels in single canine myocytes is a consequence of the cell isolation procedure.

As Shaw and Rudy (29) pointed out, the ability of K<sub>ATP</sub> channels to oppose the excitatory Na<sup>+</sup> current depends very much on the driving force for K<sup>+</sup> at the foot of the action potential, in other words, on the deviation of the resting potential (V<sub>rest</sub>) from the K<sup>+</sup> equilibrium potential (E<sub>K</sub>). Assuming, as they did, that during ischemia V<sub>rest</sub> is close to E<sub>K</sub> (and therefore the magnitude of the K<sub>ATP</sub> current to oppose the Na<sup>+</sup> current is small), the effect of K<sub>ATP</sub> activation on excitability should be limited. The difference between V<sub>rest</sub> and E<sub>K</sub> depends on the magnitude of inward leak currents present during diastole. Assuming that the ischemic K<sup>+</sup> leak is due to an increased conductance through K<sup>+</sup>-selective channels (currently the most accepted theory), Carmeliet (5) postulated the necessity of a substantial inward leak to explain the presence of
significant rate-independent $K^+$ efflux, observed even after the cessation of electrical activity (14). Note that in our experiments, Epi [$K^+]_o$, continued to rise throughout 20 min of ischemia, even though the average Epi VFR was already close to zero at 10 min of ischemia (see Fig. 2). Whereas the Endo had a much higher VFR, the level of $K^+$ accumulation was lower in the Endo than in the Epi, clearly indicating that the frequency of excursions did not influence the intensity of $K^+$ efflux.

The extent to which $V_{rest}$ during ischemia can deviate from $E_K$ remains controversial. Kleber (14) showed that these two parameters, separated by 7–8 mV under normal conditions, converge to within 1 mV after 6–7 min of ischemia. However, an intriguing study by Blake et al. (3) suggests that in the ischemic canine heart, the relationship between $V_{rest}$ and $E_K$ is frequency dependent, such that in the presence of an increased excitation rate (180 vs. 90 beats/min), depolarization of $V_{rest}$ significantly exceeds that predicted by the ischemic level of $E_K$.

Clearly, these conditions are relevant to the case of ischemic VF, where the excitation rate is initially in excess of 300–400 beats/min. Those authors showed that a $Ca^{2+}$-induced efflux had a similar effect and that the two factors combined enhanced deviation of $V_{rest}$ from $E_K$ during ischemia. This suggests a possibility that the effect of rapid activations on $V_{rest}$ during ischemia is mediated via an attendant increase in the intracellular $Ca^{2+}$ concentration. The depolarizing effects of metabolic inhibition on $V_{rest}$ were observed in spontaneously beating chick embryo myocytes (11) and adult rat ventricular myocytes subjected to cyanide (2) but not in adult mammalian ventricular myocytes subjected to a mitochondrial uncoupler (1, 22). The mechanisms of depolarizing effects of metabolic stress that are not related to hyperkalemia remain largely unknown and warrant further studies.

Conclusions. This is the first study to measure [$K^+]_o$, accumulation during ischemic VF. Comparison with published data obtained in the same species suggests that the presence of VF during ischemia has little impact on the rate of $K^+$ efflux compared with ischemia alone. Moreover, the higher level of [$K^+]_o$, in the sub-Epi than in the sub-Endo, despite the opposite distribution of the VFR, further supports the relative independence of ischemic $K^+$ efflux from excitation rate.

This study tested, and rejected, the hypothesis that the VFR decline and loss of excitability during ischemic VF in the canine heart can be explained in terms of “canonical” factors of electrical depression, hyperkalemia and $K_{ATP}$ activation, by showing a great disparity in the relationship between [$K^+]_o$, and VFR in the absence of ischemia (with or without a $K_{ATP}$ agonist), on the one hand, and the presence of ischemia, on the other hand. The alternative mechanisms of electrical depression during ischemic VF remain wide open for speculation but may include enhancement of inward leak currents, which could promote sarcolemmal depolarization beyond the level induced by extracellular $K^+$ accumulation.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: T.G.T., A.B., and A.V.Z. conception and design of research; T.G.T., P.W.V., A.B., J.S., and A.V.Z. performed experiments; T.G.T., P.W.V., A.B., J.S., and A.V.Z. analyzed data; T.G.T. and A.V.Z. interpreted results of experiments; T.G.T. and A.V.Z. prepared figures; T.G.T. and A.V.Z. drafted manuscript; T.G.T., P.W.V., A.B., J.S., and A.V.Z. edited and revised manuscript; T.G.T., P.W.V., A.B., J.S., and A.V.Z. approved final version of manuscript.

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