Regional increase in extracellular potassium can be arrhythmogenic due to nonuniform muscle contraction in rat ventricular muscle

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However, it has not yet been established what role a rise in $[K^+]_o$ plays in the initiation of these arrhythmias (7).

We have reported previously that in the myocardium with nonuniform muscle contraction, a surge of $Ca^{2+}$ occurs within the border zone between the contracting and stretched regions during the relaxation phase (47). This surge induces arrhythmogenic $Ca^{2+}$ waves and arrhythmias (38, 50). By determining their sustainability and frequency, we showed that the surges of $Ca^{2+}$ are related to the likelihood of life-threatening arrhythmias (37). During ischemia, nonuniform muscle contraction also occurs (33), particularly at the interface between the normal and ischemic regions due to a decrease in the contractile strength within the ischemic region (1). In addition, spatially nonuniform depolarization of the membrane potential occurs during ischemia due to the rise of $[K^+]_o$ within the ischemic region (7, 51). Thus, in the early stage of ischemia, the roles of nonuniform muscle contraction and/or nonuniform membrane depolarization in the occurrence of phase 1 arrhythmias have not been fully established.

Therefore, in the present study, we investigated the effects of a regional increase in $[K^+]_o$, on arrhythmogenesis using a rat trabecular model. Our results indicate that a regional increase in $[K^+]_o$, leads to nonuniform intracellular $Ca^{2+}$ waves, thereby increasing arrhythmogenesis due to nonuniform muscle contraction but not the depolarization of the membrane potential. A similar mechanism could play a role in the initiation of phase 1 arrhythmias during ischemia.

**MATERIALS AND METHODS**

Dissection and mounting of rat ventricular trabeculae. All animal procedures were performed according to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, Revised 1996) and were approved by the Ethics Review Board of Tohoku University (approval reference number: 21–98). Sprague-Dawley rats weighing 200 to 250 g were anesthetized with intraperitoneal injection of pentobarbital sodium (50 mg/kg). A total of 64 hearts were excised, and the coronary arteries were immediately perfused via the aorta with a HEPES buffered solution with 15 mmol/l KCl. Trabeculae (n = 64; length, 2.4 ± 0.2 mm; width, 241 ± 60 μm; thickness, 82 ± 4 μm in a slack condition) were dissected from the right ventricle of rats and mounted horizontally between a force transducer and a micromanipulator with a direct current torque motor, which was controlled by a personal computer, in a bath on an inverted microscope (Nikon, Japan) as described previously (37–39, 50). Preparations were equilibrated at 0.5 Hz stimulation and superfused with the HEPES buffered solution at room temperature (24°C). The composition of the HEPES solution for both dissection and perfusion was (in mmol/l) 130.8 NaCl, 5 KCl, 1.2 MgCl2, 2.8 acetate, 10 HEPES, 10 glucose, and 0.7 Na$_2$CO$_3$; pH was adjusted to 7.4 by the addition of NaOH. The solutions were equilibrated with 100% O$_2$.
Measurements of force, membrane potential, and sarcomere length. Force was measured using a silicon strain gauge (model AE-801; SenSoNor, Horten, Norway). Sarcomere length (SL) was measured using laser diffraction techniques (37–39, 50), and membrane potential was measured using ultracompartment microelectrodes as described previously (37, 38). Briefly, for the measurement of SL, the preparations were illuminated by a He-Ne laser beam (05-LHP-925; Melles Griot), and the SL was calculated from the median of the intensity distribution of the first order diffraction pattern. For the measurement of membrane potential, flexible stepped electrodes drawn with a glass microelectrode puller (Narishige, Japan) were filled with 3 mol/l KCl.

Fura-2 loading and measurement of fluorescence. [Ca2+]i in the trabeculae was assessed as described previously (39). Briefly, fura-2 pentapotassium salt (Molecular Probe, Eugene, OR) was microinjected electrophoretically into one cell and allowed to spread throughout the trabeculae via gap junctions. After the injection, the trabeculae were stimulated at 1 Hz for 30–60 min, i.e., until fura-2 had diffused uniformly throughout the preparation. Fluorescence of fura-2 from the muscle at excitation wavelengths of 340, 360, and 380 nm was filtered by a 490–530 nm band-pass filter (Nikon, Japan) and acquired using a photomultiplier tube (PMT; E1341 with a C1556 socket; Hamamatsu, Japan), which recorded the average regional fluorescence, or an image intensified charge-coupled device camera (IIC; Hamamatsu C2400-8; Hamamatsu, Japan) at 30 frames/s to assess local [Ca2+]i.

Imaging of fura-2 fluorescence. The dynamics in [Ca2+]i, were analyzed from a sequence of fura-2 fluorescence images with methods described previously (37–39, 50). The fluorescence images were converted to a sequence of 8-bit bitmap (BMP) images of 512 × 512 pixels. The pixel size in our optical system was 1.80 × 1.80 μm, and the temporal resolution was 33 ms. A fluorescence image at 360 nm in resting condition (reference image, ImRef) was sampled first, and images at 380 nm (Im380) were then recorded continuously at video rate. The overall kinetics of changes in [Ca2+]i throughout the preparation was obtained through the ratio images ImRef/Im380.

A custom made IDL program (Research Systems) was used to construct spatiotemporal representations of Ca2+ variations from Ca2+ time courses and to process the resulting digital images as follows. First, arrays of about 512 × 100–150 pixels including an image of the preparation were selected from all images at each sampling point at every 512 × 512 BMP frames. After subtraction of autofluorescence, ratio images (ImRef to Im380) were obtained from the reference array (selected from the reference image sampled at 360 nm) divided pixel by pixel to the corresponding 380-nm arrays (selected from every image sampled at 380 nm). The ratio images (ImRef to Im380) reflect the spatial distribution of [Ca2+]i in the muscle.

To assess [Ca2+]i dynamics during triggered arrhythmias, we analyzed the longitudinal distribution in [Ca2+]i, along the muscle as described previously (37–39, 50). Briefly, longitudinal profiles of pixel ratios were computed across each individual ratio image (ImRef to Im380) as follows: 1) a region of interest (ROI) was chosen over the long axis of the preparation in the first ratio image of the sequence; 2) the profile of ratios along the long axis of the trabecula was calculated by averaging the values of pixels within the ROI vertically across the muscle; and 3) ratio profiles were computed for every ratio image of the sequence by using an identical ROI and pixel averaging procedure. Finally, individual ratio profiles were plotted as a function of time. After the ratio profiles were converted to [Ca2+]i, profiles as described below, the final two-dimensional representations reflecting the spatio-temporal distribution of [Ca2+]i, along the ROI were plotted.

Calculation of [Ca2+]i. To calculate [Ca2+]i, from the ratio profiles, we used the time course of the ratio (R) obtained by the standard spectrometric method using a PMT, where R was calculated by dividing the fluorescent intensity (after subtraction of the autofluorescence of the muscle) at 340 nm excitation by that at 380 nm excitation. Consistent with results of our previous study (37–39, 50), the simultaneous use of IIC together with PMT during electrically stimulated twitch of the trabeculae confirmed that ratios calculated from digital images (ImRef to Im380) at each sampling point were closely and linearly correlated (r > 0.95) with the R obtained using the PMT. The R was converted to [Ca2+]i using the following equation: [Ca2+]i = Kd × β × (R - Rmin)/(Rmax - R), where Kd is the effective dissociation constant, Rmin is R at zero [Ca2+]i, Rmax is R at a saturating [Ca2+]i, and β is the ratio of fluorescence value for [Ca2+]i-free dye to the fluorescence value for [Ca2+]i-bound dye at 380 nm excitation. Regional [Ca2+]i, in the ratio profile taken from the fluorescent images was calculated using the [Ca2+]i, calibration of the PMT and the linear relation between the ratios observed with IIC and R with PMT.

To calculate the velocity of Ca2+ waves, we identified the peak of a Ca2+ transient during the Ca2+ wave at each pixel along trabeculae and plotted the time of the maximum against the position of the peak. The velocity was calculated from the slope of the fitted line to the plot, when regression analysis showed a linear relationship (r ≥ 0.9), as described previously (39).

Local superfusion of trabeculae. Local superfusion of trabeculae was performed as described previously (37, 38, 50). In brief, a restricted region of the trabecula was exposed to a narrow “jet” of solution (0.06 ml/min) using a glass pipette with a right-angled tip (50–100 μm diameter). The glass pipette was positioned in the perfusion bath perpendicular to the long axis of the muscle. The jet solution was usually in contact with the muscle in an area 200–300 μm, whereas the diameter of the jet before hitting the muscle was 100–150 μm. When the preparation was stimulated, the jet at the other side of the muscle moved, indicating the physical contact between the jet solution and the contracting muscle. The jet was still coherent behind the muscle without affecting other segments of the muscle, and its solution was discarded with the “bulk” solution perfusing the bath.

Increase in [K+]. To increase [K+], “regionally,” a restricted region of a trabecula was exposed to a small jet of standard HEPES solution containing 30 mmol/l KCl. The Ca2+ in the jet solution was the same concentration as that of the HEPES solution used for superfusion. To increase [K+], “globally,” the K+ concentration in the HEPES bath solution was increased from 5 to 30 mmol/l so that the entire trabecula was exposed to the high K+ concentration. Under these conditions, a spatially nonuniform distribution of [K+]i, was induced in the nonjet trabecula. The present study were thin and had less than five layers of myocytes (20).

Trabeculae superfused with standard HEPES solution containing 5 mmol/l KCl without a regional or a global increase in [K+]i, were defined as the “control” throughout the measurements in the present study.

Experimental protocol. Twitch contractions lasting more than 10 s after the cessation of electrical stimulation were defined as “sustained arrhythmias.” We used the velocity of Ca2+ waves (45) and changes in force (FCw) and [Ca2+]i, (CaCW) within almost the entire trabecula during Ca2+ waves induced with electrical stimulation as predictors of arrhythmias (31). The intervals of FCw and CaCW were defined as the time intervals between the last stimulus pulse of the electrical train and the peaks of FCw and CaCW, respectively.

First of all, to investigate whether the 30 mmol/l KCl jet can cause spatial nonuniformity of the membrane potential, [Ca2+]i, and muscle contraction, we measured membrane potential, [Ca2+]i, and sarcomere length during electrical stimulation at 400-ms intervals within the region exposed to the 30 mmol/l KCl jet (InJet) and the region 1 mm apart from the InJet (OUTJet) (n = 14), as shown in Fig. 1A. In addition, in the presence of both 3 μmol/l SEA0400 (Taisho Pharmaceutical, Tokyo, Japan), an Na+–Ca2+ exchange blocker (36), and 10 μmol/l cilididine (Ajinomoto Pharmaceuticals, Tokyo, Japan), an L/N-type Ca2+ channel blocker (13), [Ca2+]i, was measured during electrical stimulation within the InJet and OUTJet (n = 6).

Second, in the presence of 200 mmol/l isoproterenol, arrhythmias were induced by electrical stimuli at intervals of 400 ms for 30 s during a regional or global increase in [K+]i, with (n = 9) and without 10 μmol/l blebbistatin (Sigma-Aldrich, St. Louis, MO), a myosin II
ATPase inhibitor (n = 22) (16, 44). Spatial changes in [Ca\(^{2+}\)] were then recorded during sustained arrhythmias (n = 6).

Third, trains of electrical stimuli of 7.5-s duration at 400-ms intervals were repeated every 15 s in the absence of isoproterenol since the induction of arrhythmias with isoproterenol made the analysis of their triggering mechanism difficult (n = 13). The amplitude of FC\(_{\text{CW}}\) (\(\Delta F_{\text{CW}}\)) and that of Ca\(_{\text{CW}}\) (\(\Delta \text{Ca}_{\text{CW}}\)) were measured under the condition of a regional increase or a global increase in \([K^+]_o\). To further investigate the triggering mechanism of arrhythmias with the regional \([K^+]_o\), \(\Delta F_{\text{CW}}\) and \(\Delta \text{Ca}_{\text{CW}}\) were measured during the regional increase in the presence of 1) both 3 \(\mu\)mol/l SEA0400 and 10 \(\mu\)mol/l cilnidipine (n = 6), 2) 100 \(\mu\)mol/l streptomycin, a stretch-activated channel blocker (n = 7) (19), 3) 20 \(\mu\)mol/l 2,3-butanedione monoxime (BDM; n = 6) (3), and 4) 10 \(\mu\)mol/l blebbistatin (n = 5). Furthermore, spatial changes in [Ca\(^{2+}\)] were recorded during the regional increase in \([K^+]_o\) (n = 10).

All the measurements were performed within 10 min after the application of the 30 mmol/l KCl jet or the superfusion of solution containing 30 mmol/l KCl ([Ca\(^{2+}\)] \(= 3.0 \text{ mmol/l; SL}, 2.0 \mu\text{m; temperature, 24°C})).

Statistics. All measurements were expressed as means ± SE. Statistical analysis was performed using a paired t-test when the data were normally distributed. Otherwise, the Wilcoxon signed-ranks test was used. These analyses were performed using software for statistical analysis (Ekuseru-Toukei 2010; Social Survey Research Information, Tokyo, Japan). Values of \(P < 0.05\) were considered to be significant.

All the authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

RESULTS

Nonuniformity during a regional increase in \([K^+]_o\). As a result of a regional increase in \([K^+]_o\), the resting membrane potential was more depolarized and the action potential duration at -30 mV (APD\(_{\text{30}}\)) was shortened. Furthermore, spatial changes in [Ca\(^{2+}\)] were larger within the IN\(_{\text{jet}}\) versus these same values within the OUT\(_{\text{jet}}\) (Fig. 1, B and C). Thus there was spatial nonuniformity of the membrane potential along the trabecula. Concomitantly, peak [Ca\(^{2+}\)] was smaller and diastolic [Ca\(^{2+}\)] at the moment of electrical stimuli was larger within the IN\(_{\text{jet}}\) (Fig. 1, B and C). Thus there was nonuniformity of [Ca\(^{2+}\)] along the trabecula. Furthermore, as shown in Fig. 1B, sarcomeres within the IN\(_{\text{jet}}\) shortened early during
twitch and then were stretched passively by the contraction of cells within OUTjet. Sarcomeres within the OUTjet exhibited only shortening during twitch, exhibiting spatially nonuniform muscle contraction within the trabecula. To investigate whether membrane potential plays a role in the formation of nonuniform \([\text{Ca}^{2+}]\), observed, the main pathways for the \(\text{Ca}^{2+}\) efflux and influx via cell membrane, that is, \(\text{Na}^{+}-\text{Ca}^{2+}\) exchanger and \(\text{Ca}^{2+}\) channels, were blocked. In the presence of both 3 \(\mu\)mol/l SEA0400 and 10 \(\mu\)mol/l cilnidipine, peak and diastolic \([\text{Ca}^{2+}]\) within the INjet did not differ from those within the OUTjet (Fig. 1D). Thus the nonuniform \([\text{Ca}^{2+}]\) during the regional increase in \([\text{K}^{+}]\) was secondary to the nonuniform membrane potential.

Differences in inducibility of arrhythmias due to the increased spatial pattern of \([\text{K}^{+}]\). To investigate the effects of different spatial patterns of \([\text{K}^{+}]\), on the initiation of arrhythmias, trabeculae were stimulated electrically during the regional and global increase in \([\text{K}^{+}]\), in the presence of 200 nmol/l isoproterenol. Here, the regional and global increase in \([\text{K}^{+}]\) decreased the developed force to 71.7 \(\pm\) 5.5\% (\(n = 10\)) and 26.4 \(\pm\) 1.8\% (\(n = 7\)) of the values before their \([\text{K}^{+}]\) increase, respectively.

During the regional increase in \([\text{K}^{+}]\), sustained arrhythmias were induced in 10 of 14 trabeculae (Fig. 2, A and C). The amplitude of \(\text{Ca}_{\text{st}}^{2+}\) during the regional increase in \([\text{K}^{+}]\) could not be determined due to interference of arrhythmias, although...
its ascending slope was steeper than that without the regional increase (Fig. 2A, left). The spatial changes in [Ca$^{2+}$]$_o$ observed during the regional increase in [K$^+$]$_o$ represented Ca$^{2+}$ surges around the IN$_{jet}$ during the relaxation phase of muscle contraction and always preceded the synchronous increases in [Ca$^{2+}$]$_i$ of twitch contractions (Fig. 2A, right). In contrast, no arrhythmias were observed in eight trabeculae subjected to the global increase in [K$^+$]$_o$ (Fig. 2, B and C). These results suggest that the regional but not the global increase in [K$^+$]$_o$ causes arrhythmias. Furthermore, the surges of Ca$^{2+}$ within the region showing nonuniform [Ca$^{2+}$]$_i$ and contraction could play a role in arrhythmia occurrence.

To further investigate the direct effect of nonuniform contraction on the induction of these arrhythmias, trabeculae were stimulated electrically in the presence of 10 mmol/l blebbistatin as well as 200 nmol/l isoproterenol. The presence of blebbistatin decreased developed force by electrical stimulation to 5.4 ± 1.0% ($n = 9$) of the predrug values and minimized the contractile differences along the trabeculae. Interestingly, despite the presence of isoproterenol, no arrhythmias were observed in nine trabeculae during the regional increase in [K$^+$]$_o$ in the presence of blebbistatin (Fig. 2C), suggesting that by uncoupling excitation-contraction coupling, the conditions that favor arrhythmias were removed.

Ca$_{CW}$ and velocity of Ca$^{2+}$ waves during the regional increase in [K$^+$]$_o$. To investigate how arrhythmias were induced during the regional increase in [K$^+$]$_o$, FC$_W$ and Ca$_{CW}$ were determined in the absence of isoproterenol because interference of arrhythmias with isoproterenol made their measurement difficult. The regional and global increase in [K$^+$]$_o$ decreased developed force to 60.3 ± 3.7% ($n = 11$) and 30.4 ± 4.4% ($n = 6$) of the values before their [K$^+$]$_o$ increase, respectively. During the regional increase in [K$^+$]$_o$, an early FC$_W$ and an early, large Ca$_{CW}$ were induced by electrical stimulation at 400-ms intervals for 7.5 s (Fig. 3A). In contrast, with the global increase in [K$^+$]$_o$, a small FC$_W$ and an early, small Ca$_{CW}$ were induced (Fig. 3B). Because it has been reported that an early, large Ca$_{CW}$ is important for the initiation of triggered arrhythmias (31), we assumed that this enhancement of Ca$_{CW}$ during the regional increase in [K$^+$]$_o$ was involved in the initiation of arrhythmias observed in Fig. 2, A and C.

To determine whether the spatially nonuniform [Ca$^{2+}$]$_i$ was related to the enlarged Ca$_{CW}$ observed during the regional increase in [K$^+$]$_o$ (Fig. 3A), spatial changes in [Ca$^{2+}$]$_i$ were determined. As shown in Fig. 4A, the Ca$^{2+}$ surfed around the IN$_{jet}$ (arrowheads) after the electrical stimulation during the regional increase in [K$^+$]$_o$ and propagated as Ca$^{2+}$ waves through the trabeculae. Without the regional increase in [K$^+$]$_o$, Ca$^{2+}$ waves propagated slowly and sometimes could not be induced (Fig. 4, A and B). This suggests that acceleration of Ca$^{2+}$ waves underlies the enlarged Ca$_{CW}$ observed during the regional increase in [K$^+$]$_o$.

**Fig. 3. Effect of the regional (R) and global increase in [K$^+$]$_o$, (G) on the aftercontractions (FC$_W$) and [Ca$^{2+}$]$_i$ (Ca$_{CW}$) during the aftercontractions in the absence of isoproterenol.** $\Delta$FC$_W$ and $\Delta$Ca$_{CW}$ indicate the amplitude of the FC$_W$ and that of Ca$_{CW}$, respectively. Arrows with ST indicate the moments of electrical stimulation. **A.** Left: representative recordings of force (top) and average [Ca$^{2+}$]$_i$ within the entire trabecula (bottom) during the last 3 electrical stimuli (400-ms stimulus interval) with (black lines, R) and without the regional increase in [K$^+$]$_o$, (gray lines, C) ([Ca$^{2+}$]$_o$ = 3 mmol/l, temperature = 23.7°C, Experiment Number 110221). Right (4 panels): summary data of the effect of the regional increase in [K$^+$]$_o$, on the FC$_W$ and Ca$_{CW}$. At top (2 panels), the $\Delta$FC$_W$ ($n = 11$) and $\Delta$Ca$_{CW}$ ($n = 7$). At bottom (2 panels), the intervals of the FC$_W$ and Ca$_{CW}$ with (black symbols, R) and without the regional increase in [K$^+$]$_o$, (gray symbols, C), $\#P < 0.01$ vs. C (paired t-test); $\&P < 0.05$ vs. C (Wilcoxon signed-ranks test). **B.** Left: representative recordings of force (top) and average [Ca$^{2+}$]$_i$ within the entire trabecula (bottom) during the last 3 electrical stimuli (400-ms stimulus interval) with (black lines, G) and without a global increase in [K$^+$]$_o$, (gray lines, C). *P < 0.05 vs. C (paired t-test).
To minimize the \([\text{Ca}^{2+}]_i\) spatial gradients produced by nonuniform membrane depolarization along the trabeculae (Fig. 1, C and D), both 3 \(\mu\)mol/l SEA0400 and 10 \(\mu\)mol/l cilnidipine were added. In the presence of both drugs, the regional increase in \([\text{K}^+]_o\), resulted in both a small FCW and a small CACW (Fig. 5A), similar to those observed during the global increase in \([\text{K}^+]_o\) protocol (Fig. 3B). In contrast, the early FCW and the early, large CACW during the regional increase in \([\text{K}^+]_o\) (Fig. 3A) remained unchanged in the presence of streptomycin (Fig. 5B), suggesting that stretch-activated channels are not related to the measured changes in the FCW and CACW.

Finally, to determine whether nonuniform muscle contraction is related to the enhancement of CACW shown in Fig. 3A, 20 mmol/l BDM or 10 \(\mu\)mol/l blebbistatin was add to minimize the contractile differences along the observed trabecula. In the presence of BDM or blebbistatin, developed force by electrical stimulation decreased to 2.7 \(\pm\) 0.7% \((n = 6)\) or 10.9 \(\pm\) 1.0% \((n = 5)\) of the predrug values, respectively. In the presence of BDM or blebbistatin, the regional increase in \([\text{K}^+]_o\) did not accelerate \(\text{Ca}^{2+}\) waves anymore, resulting in a small CACW (compare Fig. 5, C and D, with Fig. 3A). These changes are again similar to those observed during the global increase in \([\text{K}^+]_o\) (Fig. 3B) and suggest that blebbistatin suppresses the induction of arrhythmias during the regional increase in \([\text{K}^+]_o\), despite the presence of isoproterenol, as shown in Fig. 2C. Thus, during the regional increase in \([\text{K}^+]_o\), the nonuniformity of \([\text{Ca}^{2+}]_i\) and contraction but not the depolarization of the resting membrane potential accelerates the potentially arrhythmogenic \(\text{Ca}^{2+}\) waves and enhances the CACW, causing the arrhythmias observed in Fig. 2.

**DISCUSSION**

The present study characterized the effects of a regional or a global increase in \([\text{K}^+]_o\) on arrhythmogenesis in intact rat trabeculae. To the best of our knowledge, it shows for the first time that a regional but not a global increase in \([\text{K}^+]_o\) increases arrhythmogenesis, probably due to spatially nonuniform \([\text{Ca}^{2+}]_i\) and nonuniform muscle contraction, which causes dissociation of \(\text{Ca}^{2+}\) from the myofilaments, particularly at the interface between the shortening and stretched region.

**Nonuniformity of membrane potential, \([\text{Ca}^{2+}]_i\), and muscle contraction.** The observation that the 30 mmol/l KCl jet depolarized the resting membrane potential and shortened the action potential duration within the INjet (Fig. 1, B and C) is consistent with past reports (9, 28). This regional change in membrane potential resulted in spatial nonuniformity of membrane potential as observed during ischemia (32). It has been reported that depolarization of the membrane potential decreases the net influx of \(\text{Ca}^{2+}\) through a \(\text{Na}^+\)-\(\text{Ca}^{2+}\) exchanger (40) and that the shortened action potential reduces the \(\text{Ca}^{2+}\) influx through the \(\text{Ca}^{2+}\) channel (6). Thus disappearance of nonuniformity of \([\text{Ca}^{2+}]_i\) after the inhibition of both the \(\text{Na}^+\)-\(\text{Ca}^{2+}\) exchanger by
Regional application of a high \([\text{K}^+]_\text{o}\) jet resulted in an early, large \(\Delta \text{Ca}_{\text{CW}}\) (Fig. 3A), accelerated the arrhythmogenic \(\text{Ca}^{2+}\) waves (Fig. 4), and induced sustained arrhythmias in the presence of isoproterenol (Fig. 2, A and C). These observations suggest that arrhythmias during the regional increase in \([\text{K}^+]_\text{o}\) were triggered by enhancement of delayed afterdepolarizations (DADs) since in the endocardium (35), the earlier and larger occurrence of \(\text{Ca}_{\text{CW}}\) reflects larger DADs (20) and the velocity of \(\text{Ca}^{2+}\) waves correlates with the amplitude of DADs (45). In addition, the early, large \(\Delta \text{Ca}_{\text{CW}}\) and the accelerated \(\text{Ca}^{2+}\) waves during the regional increase in \([\text{K}^+]_\text{o}\) are caused by nonuniform \([\text{Ca}^{2+}]_i\), and contraction for the following three reasons. First, a global increase in \([\text{K}^+]_\text{o}\) decreased the \(\Delta \text{Ca}_{\text{CW}}\) in the absence of isoproterenol (Fig. 3B), and sustained arrhythmias were not inducible in the presence of isoproterenol (Fig. 2, A and C). Second, after minimization of nonuniform \([\text{Ca}^{2+}]_i\), by the inhibition of both the \(\text{Na}^+\)-\(\text{Ca}^{2+}\) exchanger and the \(\text{Ca}^{2+}\) channels, the \(\Delta \text{Ca}_{\text{CW}}\) decreased during the regional increase in \([\text{K}^+]_\text{o}\) (Fig. 5A). Third, after minimization of nonuniform contraction by the inhibition of muscle contraction, the \(\Delta \text{Ca}_{\text{CW}}\) decreased and the velocity of \(\text{Ca}^{2+}\) waves did not increase anymore during the regional increase in \([\text{K}^+]_\text{o}\) (Fig. 6, C and D), and sustained arrhythmias were never induced in the presence of isoproterenol (Fig. 2C). Taken together, these results suggest that the regional increase in \([\text{K}^+]_\text{o}\) enhanced arrhythmogenesis through spatially nonuniform \([\text{Ca}^{2+}]_i\), and nonuniform muscle contraction.

As for the initiation of \(\text{Ca}^{2+}\) waves, two mechanisms have been reported. One is SR \(\text{Ca}^{2+}\) leak due to \(\text{Ca}^{2+}\) overload (47), and the other is \(\text{Ca}^{2+}\) dissociation from the myofilaments in the myocardium with nonuniform contraction (37, 38, 50). In the myocardium with nonuniform contraction, regional differences in contractile strength may cause paradoxical stretching and shortening of the impaired muscle by contractions of the more viable neighboring muscle. During the relaxation of the neighboring muscle, \(\text{Ca}^{2+}\) is dissociated from the myofilaments due to the paradoxical shortening of the impaired muscle, induces additional \(\text{Ca}^{2+}\) release from the SR (38), and thus forms larger initiators of \(\text{Ca}^{2+}\) waves (\(\text{Ca}^{2+}\) surges) (50). \(\text{Ca}^{2+}\) waves propagate faster by this larger initiator (39) and cause triggered arrhythmias (37). Ter Keurs et al. (46) named this sequence of events reverse excitation-contraction coupling (RECC). In the present study as well, the \(\text{Ca}^{2+}\) surged around the INjet during sustained arrhythmias (Fig. 2A) and propagated as \(\text{Ca}^{2+}\) waves during the regional increase in \([\text{K}^+]_\text{o}\) (Fig. 4). Thus it is probable that in the present study, arrhythmias occurred through RECC.

Clinical implications. After the onset of ischemia, \([\text{K}^+]_\text{o}\) increases to more than 20 mmol/l (7, 51) due to \(I\) increased \(\text{K}^+\) efflux through ATP-dependent \(\text{K}^+\) channels (4, 27, 41), decreased active \(\text{K}^+\) influx through \(\text{Na}^+/\text{K}^+\) pump (41, 48), and ...
and 3) shrinkage of extracellular space. This rise in \([K^+]_o\) shows a triphasic pattern (7), which overlaps that of the occurrence of phase 1 arrhythmias. Some of the known electrophysiological effects of acute ischemia can be mimicked by the rise in \([K^+]_o\) (22, 23), and alteration of \([K^+]_o\) due to modulation of ATP-dependent K⁺ channels affects the occurrence of arrhythmias (17, 29). Based on these findings, it has been proposed that the rise in \([K^+]_o\) is strongly related to phase 1 arrhythmias. In addition, it has been reported that an elevation of catecholamine also occurs 10 min after the onset of ischemia (43). Thus it is reasonable to infer that the mechanisms of arrhythmias during the early phase of ischemia are similar to those studied here.

As for mechanisms underlying phase 1 arrhythmias in animal models, several candidates such as re-entry (2, 30), triggered activity (49) due to gap junctional uncoupling (15), and mechanically induced membrane depolarization (26) have been reported. Here, we propose that the Ca²⁺ dissociated from the myofilaments within the border zone between the contracting and stretched region (37, 38, 50) is also involved in the occurrence of phase 1 arrhythmias since nonuniform muscle contraction actually occurs at the interface between normal and ischemic tissue during acute ischemia (33).

Interestingly, it has been reported that the incidence of phase 1 arrhythmias is related to left ventricular wall stress (8), which may affect the regional differences in contractile strength in the myocardium during nonuniform contraction. Additionally, Gd³⁺, a stretch-activated channel blocker, does not suppress phase 1 arrhythmias (5) in the same way that streptomycin did not affect arrhythmogenesis in the present study (Fig. 5B). Furthermore, it has been reported that Ca²⁺ channel blockers prevented the onset of arrhythmias during ischemia (12, 18) in the same way that cilnidipine played a role in the disappearance in nonuniform [Ca²⁺]i in the present study (Figs. 1C and 5A). Thus the reports in the past are not contradictory to the hypotheses proposed in the present study.

In conclusion, results of the present study show that a regional but not a global increase in \([K^+]_o\) increases arrhythmogenesis due to nonuniform [Ca²⁺]i and muscle contraction, which causes Ca²⁺ to dissociate from the myofilaments, particularly at the interface between the shortening and stretched region. The same mechanism may also be important for the occurrence of arrhythmias during the early phase of ischemia.

Study limitations. Acute myocardial ischemia causes redistribution of a number of ions across the cell membrane, including net \([K^+]\), loss and subsequent \([K^+]\), accumulation (4, 41, 48). In the present study, however, only the effects of the regional increase in \([K^+]\) were measured. Thus this model does not precisely mimic electrophysiological consequences due to the influence of acute ischemia on the activity of a variety of ion channels and transporters (10, 14, 42). Nevertheless, the results in the present study are still important because it shows for the first time that a regional increase in \([K^+]\) causes arrhythmias by itself, probably due to spatially nonuniform [Ca²⁺]i and nonuniform muscle contraction.

**REFERENCES**


