Genesis of life-threatening ventricular arrhythmias during the delayed phase of acute myocardial ischemia. Role of cellular electrical coupling and myocardial heterogeneities

de Groot, J.R.

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METABOLIC AND ELECTROPHYSIOLOGICAL DIFFERENCES BETWEEN ISOLATED SINGLE CELLS AND CELL PAIRS IN RESPONSE TO METABOLIC INHIBITION IN NORMAL AND HYPERTROPHIC RABBIT LEFT VENTRICULAR MYOCYTES


Submitted for publication

Summary.

Introduction. Ischemia-induced cellular uncoupling creates an arrhythmogenic substrate in multicellular myocardial preparations. We hypothesized that intrinsic metabolic differences among myocytes are enhanced during hypertrophy and underlie the prolonged duration of the process of cellular uncoupling. Intact cellular coupling masks these differences.

Methods and results. In isolated left ventricular myocytes from control and hypertrophic rabbit hearts and in side-to-side coupled cell pairs we investigated terminal rise in \([\text{Ca}^{2+}]\), time to rigor and action potentials during metabolic inhibition. Terminal calcium rise occurred after 12 to 27 minutes, and closely preceded rigor. Differences in anaerobic reserve among cells cause this variability. In 11/15 cell pairs, rigor developed in the second cell within one minute from the first, and coupling remained intact up to rigor. No baseline differences were found in calcium metabolism or action potential duration in hypertrophic versus control myocytes, however, terminal calcium rise occurred significantly earlier than in control cells (15.3±5.9 vs 17.0±3.7 minutes), and variability in time to rigor was larger (12.6 vs 101 minute, p<0.001). Action potentials tended to shorten less in hypertrophic cells, and \([\text{Ca}^{2+}]\), was higher during metabolic inhibition, leading to earlier rise in \([\text{Ca}^{2+}]\).

Conclusions. The duration of cellular uncoupling in intact preparations can be explained by large variability in terminal calcium rise and time to rigor in single cells. This variability is caused by different anaerobic reserve among cells, enhanced during hypertrophy and masked through intact cellular coupling. Cellular coupling remains intact until the moment of rigor, which makes the existence of electrophysiological heterogeneities between adjacent cells unlikely.
Introduction.

Ischemia-induced loss of cellular communication during the course of the cellular uncoupling process has been associated with the occurrence of lethal ventricular arrhythmias. The incidence and severity of ventricular arrhythmias and sudden death during acute ischemia is enhanced in left ventricular hypertrophy. The duration of ischemia induced cellular uncoupling is increased in failing hearts with combined volume and pressure overload. In addition, gap junction density is decreased. For arrhythmias to occur, large heterogeneities need to be present. When cellular coupling is intact, it is unlikely that such heterogeneities are exposed, because cellular coupling provides intercellular equilibration of ionic homeostasis and energy metabolism, and consequently of action potential duration, because ions and small molecules pass gap junctions easily. Ischemia induced cellular uncoupling might disturb this equilibration and thus unmask the intrinsic heterogeneities in cardiac tissue.

We hypothesize that intrinsic metabolic and electrophysiological differences are larger in left ventricular hypertrophy than in normal hearts. Cellular uncoupling during acute ischemia might unmask these intrinsic heterogeneities and create an arrhythmogenic substrate. Therefore, we tested the response to metabolic inhibition of a population of single left ventricular myocytes isolated from normal and hypertrophic hearts with respect to the terminal rise in diastolic calcium and the occurrence of rigor, which have been shown to closely precede cellular uncoupling in intact preparations. In addition, we tested whether metabolic and electrophysiological differences are larger in normal myocytes than in coupled cell pairs.

Methods.

Induction of left ventricular hypertrophy and isolation of left ventricular myocytes

Left ventricular hypertrophy was induced in 6 rabbits as described in chapter 2. From those animals, and from 16 control rabbits, left ventricular myocytes were isolated as described in chapter 2.

Experimental setup and measurement of cytosolic calcium

Handling of myocytes and intracellular calcium measurements have been described previously. After control recordings were obtained, myocytes were superfused with normoxic HEPES solution, containing 2.6 mmol/l Ca²⁺, 2 mmol/l NaCN and no glucose. Thirty-eight hypertrophic myocytes were studied. Cell dimensions were measured with a vernier in 250 hypertrophic and 200 control myocytes. Thirty-eight control single myocytes and 15 cell pairs were investigated. In another 32 control myocytes glycolysis was blocked by adding 1 mmol/l iodoacetate to the HEPES solution with NaCN. By blocking glycolysis the contribution of the anaerobic reserve, defined as the glycogen content, on differences in time to rigor among cells could be investigated. To compare differences in time to rigor between two electrically coupled paired cells with single isolated myocytes, we determined differences in time to rigor between the arbitrarily chosen groups of even and odd numbered single myocytes.

During the application of metabolic inhibition, 5 second recordings of Indo-1 fluorescence were taken every 2 minutes. Myocyte shape was monitored visually between recordings. Occurrence of rigor was defined as the rapid transition from rod shaped to square or round shaped which occurred within seconds.
Recordings were analyzed offline as described in chapter 2. The moment of terminal rise in cytosolic calcium was arbitrarily defined as a 10% increase in diastolic calcium over the mean diastolic calcium during the first 10 minutes of metabolic inhibition. In 6 control myocytes exact timing of rigor was not possible because rigor took place during recording of the Indo-1 fluorescence. In addition, time to terminal calcium rise could not be determined accurately in 3 hypertrophic and 1 control myocyte. These data are not included in the comparisons.

**Electrophysiological recordings**

Action potentials from 12 hypertrophic myocytes, 10 control myocytes and 5 control cell pairs were measured with the perforated patch clamp technique at 37°C. Details are described in chapter 2.

**Statistics**

Unless stated otherwise, data are presented as mean±SD. To determine differences between groups, an unpaired t-test was used. When data were not normally distributed, a Mann-Whitney test was used as a non-parametric alternative. Differences in variability were determined with an F-test. Significance of correlations was tested with Pearson’s product moment correlation. For multiple comparisons ANOVA for repeated measures was used. A p<0.05 indicated statistical significance.

**Results.**

Myocytes isolated from rabbits with ischemia-induced hypertrophy were significantly larger

| Table 6.1 Baseline characteristics of control and hypertrophic myocytes |
|-----------------|-----------------|----------|
|                | Control | Hypertrophy | p=   |
| **Cell dimensions** | n=200  | n=250  |       |
| Cell length (µm) | 137±25  | 166±37  | <0.001|
| Cell width (µm)  | 28±9    | 35±10   | <0.001|
| **Action Potential Characteristics (2 Hz)** | n=10 | n=12 |       |
| APD90 (ms)       | 235±55  | 239±27  | NS    |
| APD50 (ms)       | 185±57  | 197±34  | NS    |
| Vmax (V/s)       | 116±20  | 109±34  | NS    |
| MDP (mV)         | -76±4   | -76±3   | NS    |
| APA (mV)         | 118±4   | 118±9   | NS    |
| **Calcium measurements** | N=38 | n=38 |       |
| [Ca²⁺] diastolic (nmol/l) | 97±30  | 92±37   | NS    |
| [Ca²⁺] systolic (nmol/l)  | 270±109 | 244±100 | NS    |
| Ca²⁺ transient amplitude (nmol/l) | 173±90 | 152±77 | NS    |

APD90: action potential duration at 90% repolarization, APD50: action potential duration at 50% repolarization, Vmax: maximal upstroke velocity, MDP: mean diastolic potential, APA: action potential amplitude.
than control myocytes (table 6.1). Before metabolic inhibition, no differences between hypertrophic and control myocytes were found with respect to calcium metabolism and action potential characteristics (table 6.1). In these rabbits with left ventricular hypertrophy clinical and postmortem signs of heart failure were absent

Metabolic inhibition in control cardiomyocytes

Figure 6.1, panel A shows a representative example of sequential calcium transients recorded during metabolic inhibition. Following application of NaCN, the calcium transient amplitude progressively decreased. In this example, transients entirely disappeared after 16 minutes, and diastolic calcium started to rise. After 18 minutes, this cell developed rigor. Panel B shows the time course of change of systolic and diastolic calcium concentrations in the same cell.

There was a large variation in the moment of terminal calcium rise and rigor among individual cells (table 6.2). Terminal rise in calcium preceded rigor by 1.2±2.1 minutes (n=32, r=0.83, p<0.001). Figure 6.2 shows the cumulative representation of time of rise in calcium and onset rigor in 32 control cells from which both time to terminal calcium rise and time to rigor were available. Table 6.2 displays the average time to terminal calcium rise and rigor. These data confirm the close coupling between rise in calcium and rigor, and closely resemble the time course of uncoupling and calcium rise in multicellular preparations.

To evaluate whether the variability in tolerance to metabolic inhibition between individual myocytes could be the consequence of a difference in anaerobic reserve, we blocked the anaerobic glycolysis in 32 myocytes with iodoacetate 1 mM added to the NaCN containing HEPES solution. In these myocytes, rigor developed after 4.2±0.5 minutes (p<0.001 vs metabolic inhibition alone) and the variation in time to rigor was significantly reduced in comparison with the cells that underwent metabolic inhibition with NaCN alone (0.26 and 12.6 minutes respectively, F-test: p<0.001). The difference remained significant after normalization to the mean time of rigor (0.01 compared to 0.04 in control cells, F-test: p<0.01). Hence, the variability observed in tolerance to metabolic inhibition can be most likely attributed to differences in anaerobic reserve, probably the amount of glycogen storage in individual cells.

Figure 6.3, panel A shows a representative example of the course of action potential shortening in a single isolated myocyte. This action potential progressively shortened by more than 80% after 18 minutes of metabolic inhibition, after which the cell became inexcitable (table 6.2). Panel B shows the course of action potential shortening during metabolic inhibition in 10 control cells. Mean time of inexcitability did not differ significantly from time to terminal calcium rise or rigor (table 6.2). Panel C shows the cumulative representation of time to inexcitability in 10 myocytes. Note the resemblance with figure 6.2.

<table>
<thead>
<tr>
<th>Table 6.2 Metabolic inhibition in control and hypertrophic myocytes</th>
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<tr>
<td><strong>Control</strong></td>
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<tr>
<td>Time to inexcitability (min)</td>
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<td>Time to terminal [Ca²⁺] rise (min)</td>
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<td>Time to rigor (min)</td>
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*Terminal [Ca²⁺] rise: 10% increase over mean diastolic [Ca²⁺] during the first 10 minutes metabolic inhibition. Rigor: visual contracture. *p<0.05, †p<0.01, ‡p<0.001
A: [Ca$^{2+}$] (nmol/l)

- A, 0 min
- B, 1 min
- C, 5 min
- D, 10 min
- E, 15 min

Time (msec)

B: [Ca$^{2+}$], (nmol/l)

Duration of metabolic inhibition (min)
Figure 6.1. Panel A: representative example of subsequent calcium transients during control and after 0 (A), 1 (B), 5 (C), 10 (D) and 15 (E) minutes of NaCN induced metabolic inhibition. Panel B: time course of change in systolic (open squares) and diastolic (closed circles) \( [Ca^{2+}] \) in the same cell as in panel A. Lettering as in panel A. Shortly after the transients disappear, diastolic \( [Ca^{2+}] \), starts to rise and rigor develops.

To test whether cellular coupling could equilibrate intrinsic metabolic differences between individual cells, we investigated time to terminal calcium rise and rigor in side-to-side coupled cell pairs. Although less gap junctions are present at side-to-side connections than at end-to-end junctions, side-to-side coupled cells have been demonstrated to be electrically coupled\(^{21}\).

In 11 out of 15 cell pairs investigated, the onset of rigor in one cell was followed by rigor in the other cell within one minute. Time to rigor in both of the paired cells highly correlated (\( r=0.83, p<0.0005 \)). Such correlation was absent when difference in time to rigor was compared in odd and even numbered isolated single cells (\( r=-0.123, p=NS \)). Figure 6.4 shows the difference in time to rigor in cells from all cell pairs (2.6±5.3 minutes) compared to the difference in time to rigor between odd and even numbered single cells (3.9±2.7, \( p<0.05 \) vs cell pairs). Note that in two cell pairs the delay between time to rigor in subsequent cells exceeded 10 minutes. Most likely, these cells were initially not well coupled, despite their physical connection. Nevertheless, these data demonstrate that cells originating from the same region of the heart do exhibit intrinsic functional differences.
Figure 6.3. Panel A: subsequent action potentials from a single isolated myocyte during the course of metabolic inhibition. Panel B: APD90 (mean±SEM) from 10 single myocytes. Panel C: cumulative representation of the moment of inexcitability in 10 isolated single myocytes. Note the resemblance with figure 6.2.

Figure 6.4. Time between rigor in the two paired cells and between even and odd single myocytes (p<0.05). Note that in 11/15 cell pairs rigor in the second cell developed within a minute from the first cell.

Despite that the Indo-1 fluorescence was recorded from both cells, and that the measurement of terminal calcium rise might thus be less accurate for the individual cells, time to terminal calcium rise preceded onset of rigor (first cell: r=0.47, p=0.07, second cell: r=0.65, p<0.01). These data demonstrate that in coupled cell pairs, the time to rigor is closely related for the two individual cells, indicating equilibration of their metabolism during metabolic inhibition through intact cellular coupling. This finding supports our hypothesis that intact coupling equilibrates intrinsic differences between individual cells.

Figure 6.5A shows an example of action potentials elicited in a cell pair during the course of metabolic inhibition. Panel B shows a magnification of the upstrokes of the action potentials in panel A. Note that the second cell (follower) immediately followed the first, (paced) cell without significant delay, up to the moment when the paced cell became inexcitable. Panel C shows that virtually no differences were found in the course of action potential shortening between the cells in this cell pair. Equilibration of APD between the two cells lasted until the paced cell became inexcitable. In 5 cell pairs investigated, action potential duration remained equal in both cells up to the moment of inexcitability. Obviously, it cannot be concluded from these data whether both cells become inexcitable at the same moment, because only one cell of the pair was paced. As demonstrated above, there was no difference in time to inexcitability and time to rigor.
Figure 6.5. Panel A: Subsequent action potentials during NaCN induced metabolic inhibition in a control cell pair. Both onset and duration of the action potential are equal in the paced and the follower cell. Panel B: magnification of the upstroke of the action potentials in panel A. Cells remain well coupled up to inexcitability and no delay between the cells develops. Panel C: course of action potential duration in the same cell pair as in A and B. Action potential in the follower cell remains equal to that in the paced cell up to inexcitability.

Hence, cellular coupling remains intact and undisturbed up to inexcitability, terminal calcium rise and rigor. Cellular uncoupling is a fast process at the level of the individual cell, the onset of which can be heterogeneous among individual myocytes.

Terminal $[Ca^{2+}]$, rise, rigor and action potential duration in hypertrophic cardiomyocytes

Figure 6.6 panel A shows calcium transients from a representative hypertrophic myocyte and panel B shows the systolic and diastolic calcium concentration during metabolic inhibition in the same cell. After onset of terminal calcium rise, diastolic calcium typically increased less in hypertrophic than in control myocytes, and in 22 out of 38 cells a secondary decrease in diastolic calcium was observed several minutes after calcium rise and rigor. The temporal relation between terminal calcium rise and rigor holds true in most but not all cells from hypertrophic rabbit hearts: time to terminal calcium rise preceded time to rigor in 27 out of 38 myocytes. Table 6.2 shows that terminal calcium rise occurred earlier in hypertrophic than in control myocytes ($p<0.05$). There was no difference in time to rigor. However, the statistical variance in time to rigor was significantly larger in hypertrophic myocytes than in control cells (table 6.2), indicating a larger intrinsic metabolic heterogeneity between individual hypertrophic cells.

Panel C shows the cumulative representation of time to terminal calcium rise and time to rigor in hypertrophic cells. Note the different scale on the axis compared with figure 6.2. These data demonstrate that although terminal calcium rise and rigor are related in hypertrophic cells, the time window between terminal calcium rise and rigor in hypertrophic cells exceeds that in control cells.
(6.1±11 minutes vs 1.2±2.1 minutes, p<0.05). The earlier onset and longer duration of this process is consistent with the findings in failing rabbit papillary muscles.

Action potentials (2 Hz) from 12 hypertrophic cells were investigated. Before metabolic inhibition, there was no difference in APD, AP overshoot or resting membrane potential between hypertrophic and control myocytes (table 6.1). Figure 6.7 displays the course of APD shortening of hypertrophic myocytes. During metabolic inhibition, APD shortened biphasically also in hypertrophic myocytes, but cells were able to maintain a "plateau" APD longer than control cells (p<0.05 between 7 and 12 minutes). Thus, apart from the changes in calcium handling demonstrated above, hypertrophic myocytes appear to maintain a longer action potential duration during metabolic inhibition than control cells do, despite the absence of differences before metabolic inhibition and in time to rigor. Possibly, more [Ca^{2+}] influx occurs, resulting from decreased APD shortening causing increased diastolic [Ca^{2+}]. Indeed, diastolic [Ca^{2+}], was higher in hypertrophic than in control myocytes between 9 (64.1±31.7 vs 49.8±21.1 nmol/l, p<0.05) and 13 minutes (79.6±41.5 vs 55.6±24.8 nmol/l, p<0.005).

**Discussion.**

This study reports that: 1) During metabolic inhibition single cells exhibit a large variation in the time to inexcitability, to terminal calcium rise and to rigor attributable to differences in anaerobic reserve in individual cells. 2) In electrically coupled cell pairs variation between the two cells in time to rigor is negligible and 3) until the occurrence of inexcitability and rigor, cells remain well coupled. 4) In hypertrophic myocytes terminal calcium rise occurs earlier and variation in time to rigor is larger than in control cells; duration between terminal calcium rise and rigor is increased and action potentials shorten less rapidly.

**Electrical "uncoupling" on a cellular level**

Ischemia-induced cellular uncoupling is associated with lethal arrhythmias, but it has not been demonstrated whether uncoupling is a fast but heterogeneous process or is caused by slow and progressive homogeneous closure of the gap junctions. The former case could be arrhythmogenic and could create conditions favorable for (micro) reentrant activation. However, ischemia induced microreentry has never been demonstrated in intact hearts, although it has been described in cell cultures subjected to the gap junctional uncoupler palmitoleic acid.

The terminal increase in [Ca^{2+}], followed by development of rigor, has been demonstrated to closely precede cellular uncoupling under various circumstances. We use these as an indirect measure of time of cellular uncoupling in single cells that obviously are not coupled. The relation between uncoupling and rigor is further strengthened by the observation that the free energy of ATP hydrolysis rapidly decreases with the same time course as the onset of uncoupling. Beardslee et al. showed that cellular uncoupling is associated with dephosphorylation of connexin43, which demonstrates a direct relation between energy content of the myocyte and gap junctional conductance. Large intrinsic differences in timing of electrical inexcitability, terminal rise of [Ca^{2+}], and rigor are unmasked between individual isolated single myocytes. These differences relate to the different anaerobic reserve, most likely different glycogen content, as indicated by the iodoacetate experiments. The cumulative representation of terminal calcium rise and rigor (figure 6.2) strikingly resembles the time course of cellular uncoupling and the rise in diastolic calcium in the rabbit papillary muscle, suggesting that the duration of the process of cellular uncoupling in intact tissue...
reflects an ensemble time average of uncoupling of the individual myocytes, rather than a homogeneous slow increase of gap junctional coupling resistance. In hypertrophic myocytes, the variability in time to terminal calcium rise and rigor is enhanced, reflecting larger intrinsic differences between these cells. However, in coupled cell pairs, we found a decrease of variability in time to terminal calcium rise and time to rigor. In the vast majority, rigor in the second cell developed within a minute from the first cell, which was significantly less than that between odd and even numbered single cells. We cannot exclude that in the cell pairs in which there was a large delay in time to rigor between the first and second cell, initial coupling was absent or insufficient, and that these cells were only physically connected via the insufficiently degraded extracellular matrix and not electrically coupled. These outlying values demonstrate that intrinsic variation in properties may occur between adjacent cells. Thus, the heterogeneity observed in single myocytes cannot be attributed to their origin from different regions in the heart.

Electrical coupling between both cells remains intact until inexcitability of the paced cell (figure 6.5). Since in single cells there was no difference in time to inexcitability and time to rigor, we infer that coupling remains functional until rigor. Therefore we suggest that uncoupling is a fast process and does not relate to a slow increase in gap junctional resistance but that intrinsic differences in individual cells cause heterogeneity.

Despite close metabolic and electrical coupling in cell pairs, large regional differences are found in the intact heart\textsuperscript{25,26}. Differences in action potential duration between ischemic and non-ischemic tissue for example, cause a "current of injury" to flow, but action potential duration does not completely equilibrate\textsuperscript{27}. Hence, cellular coupling is sufficient to allow equilibration of ionic and metabolic homeostasis between adjacent cells, but cannot prevent functional heterogeneities to arise over larger distances.

Therefore, and because cellular coupling persists up to the moment of inexcitability, the occurrence of microreentry during ischemia is unlikely. The substrate for macro reentrant arrhythmias might well arise over larger distances. It might be speculated that non-ischemic cells remain well coupled to ischemic cells in the lateral and transmural ischemic border zones. This could create local electrical and metabolic disturbances, providing an arrhythmogenic substrate.

Ischemia-induced left ventricular hypertrophy

In this study we present data from cells obtained from a model of ischemia-induced hypertrophy, adapted from Pye et al\textsuperscript{28}. In hypertrophy, the incidence and severity of lethal ventricular arrhythmias are increased\textsuperscript{3} and the density of gap junctions is reduced\textsuperscript{5,6}. We report a larger statistical variance in time of terminal calcium rise and time to rigor in hypertrophic myocytes compared to control cells. Given the association between cellular uncoupling and lethal ventricular arrhythmias\textsuperscript{1,4}, we suggest that during ischemia the arrhythmogenic substrate is present for a longer time in hypertrophic than in normal hearts.

Relative depletion of $[\text{Ca}^{2+}]$ of the sarcoplasmic reticulum could contribute to a better tolerance of metabolic deterioration in hypertrophic cells, but baseline differences in systolic and diastolic calcium and in calcium transient amplitude were absent, and average time of onset of rigor was similar in hypertrophic and control cells.
Action potentials in hypertrophic cells of rabbit and rat are prolonged especially at long cycle lengths, but differ only modestly at physiological frequencies. In hearts from guinea pigs with aortic constriction, the prolongation of action potential duration was restricted to subepicardium and midmyocardium, and transmural gradients were lost. We did not select myocytes from these different regions, and found no baseline differences in action potential duration (2 Hz) between hypertrophic and control myocytes. However, during the course of metabolic inhibition, hypertrophic myocytes maintained a longer action potential than control myocytes. This contradicts with previous reports during ischemia. However, unlike in ischemia, the composition of the extracellular space remains unchanged during metabolic inhibition with subsequent absence of acidosis and hyperkalemia. Vermeulen et al. demonstrated a higher $[K^+]_o$ in ischemic failing hearts than in normal hearts, which could well account for the reported increased action potential shortening during ischemia in intact failing hearts. Maintaining a longer action potential duration during metabolic inhibition allows more calcium influx, resulting in an earlier terminal calcium rise. Indeed, during the course of metabolic inhibition, $[Ca^{2+}]_o$ in hypertrophic myocytes was higher than in control between 9 and 13 minutes.

Limitations of the study

The objective of this study was to gain insight in the process of metabolic and electrophysiological equilibration through cellular coupling. The experimental model used however, allows measurement of calcium and rigor, but except for cell pairs, single cells obviously do not allow to evaluate cellular coupling. Because data from the literature state that calcium rise and rigor
always precede cellular uncoupling\textsuperscript{16}, and that cellular uncoupling is directly related to energy content of the myocyte during ischemia\textsuperscript{24}, we used the time course of change of these parameters in single cells as an indirect measure of the uncoupling process.

Conclusions

Large variability in timing of terminal [Ca\textsuperscript{2+}], rise and rigor among individual isolated ventricular myocytes can explain the duration of the uncoupling process in multicellular preparations\textsuperscript{16}. This variability is caused by differences in anaerobic reserve between individual cells, and is reduced when cells are electrically coupled, as are intrinsic differences in action potential duration. Action potentials remain longer in hypertrophic cells than in control cells and variability in time to rigor is increased during metabolic inhibition, underlining the larger intrinsic heterogeneity. The longer action potential plateau allows more Ca\textsuperscript{2+} influx than in control cells and consequently, an earlier increase in [Ca\textsuperscript{2+}]. Thus, ischemia of hypertrophic hearts may provide an arrhythmogenic substrate during a longer time than ischemia of normal hearts. Because cells are excitable up to the occurrence of rigor without delay in propagation of the action potential between neighboring cells or dissociation of action potential duration, ischemia-induced uncoupling is most likely a fast process occurring heterogeneously within different regions of the heart. Persistence of cellular coupling up to inexcitability during ischemia prevents local heterogeneities, but might allow arrhythmogenic heterogeneity over a larger distance.

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