Slow Conduction in Cardiac Tissue, I
Effects of a Reduction of Excitability Versus a Reduction of Electrical Coupling on Microconduction

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Abstract—It was the aim of this study to characterize the spread of activation at the cellular level in cardiac tissue during conduction slowing, a key element of reentrant arrhythmias; therefore, activation patterns were assessed at high spatiotemporal resolution in narrow (70 to 80 μm) and wide (230 to 270 μm) linear strands of cultured neonatal rat ventricular myocytes, using multiple site optical recording of transmembrane voltage. Slow conduction was induced by graded elevation of [K+]o, by applying tetrodotoxin, or by exposing the preparations to the gap junctional uncouplers palmitoleic acid or 1-octanol. The main findings of the study are 4-fold: (1) gap junctional uncoupling reduced conduction velocity (range, 37 to 47 cm/s under control conditions) to a substantially larger extent before block (≤1 cm/s; ultra-slow conduction) than did a reduction of excitability (range, ∼10 to 15 cm/s); (2) activation wavefronts during uncoupling meandered within the boundaries of the preparations, resulting in a pronounced additional slowing of conduction in wide cell strands; (3) at the cellular level, propagation during uncoupling-induced ultra-slow conduction was sustained by sequentially activated tissue patches, each of which consisted of a few cells being activated simultaneously; and (4) depending on the uncoupler used, maximal action potential upstroke velocities during ultra-slow conduction were either slightly (palmitoleic acid) or highly (1-octanol) depressed. Thus, depolarizing inward currents, the spatial pattern and degree of gap junctional coupling, and geometrical factors all contribute in a concerted manner to conduction slowing, which, at its extreme (0.25 cm/s measured over 1 mm), can reach values low enough to permit, theoretically, reentrant excitation to occur in minuscule areas of cardiac tissue (≪1 mm²). (Circ Res. 1998;83:781-794.)

Key Words: impulse propagation ■ discontinuous conduction ■ gap junction ■ action potential upstroke ■ voltage-sensitive dye

It is well established on the basis of conduction measurements at the macroscopic scale that the main causes of slowing of conduction, a key ingredient of reentrant arrhythmias, are (1) a reduction of excitability,1–5 (2) a decrease of gap junctional coupling,6–9 and (3) changes in the cellular architecture of cardiac tissue.10–13 Whereas these studies offered insights into the characteristics of slow conduction at the macroscopic scale, computer simulations provided the framework for analyzing the effects of either a reduction of the sodium inward current (INa) or of gap junctional coupling on conduction at the microscopic, ie, cellular and subcellular level.6,14–18 Although, in general, both experimental and theoretical studies yielded comparable results, several controversial issues remained.

First, computer simulations suggested that an isolated decrease of gap junctional coupling should reduce conduction velocity (θ) to a substantially higher degree before occurrence of block (up to −99.5%) than a reduction of INa alone (up to −70%).6,14,16,18 However, experimental studies involving acute cell-to-cell uncoupling yielded reductions of θ (−60% to −85%),6–8 which were only slightly larger or even smaller than those reported in studies involving a reduction of excitability (∼40% to −60%),14,18,19 −95% to −98%). The importance of this discrepancy relates to the question of the minimal pathlength supporting reentrant excitation at the microscopic scale (micro-reentry): whereas at θ=7 cm/s (lowest reported experimental value during acute cell-to-cell uncoupling)6 and at an action potential duration of 100 ms typical for rat ventricular myocytes,10 “micro” would signify a circle with a diameter of ≈2 mm (pathlength, 7 mm), θ of ≈1 cm/s (theoretically predicted for cell-to-cell uncoupling)8,14,17 would reduce the diameter of the circle to ≈300 μm (pathlength, 1 mm), thus permitting reentrant excitation to occur at the few-cell level. Although it has previously been observed that return beats attributed to electrotonic reflection or micro-reentry can originate in small tissue areas,21,22 the smallest-ever directly demonstrated case of reentrant excitation was observed in the absence of acute cell-to-cell uncoupling in nonuniform anisotropic myocardium during premature stimulation (pathlength, ≈6 mm).12

Second, the assumption made in most computer models of slowed conduction, namely that gap junctional uncoupling is
reduced homogeneously along a chain of cells, has escaped a direct experimental verification thus far because it was technically impossible to measure systematically the spatial pattern of activation during slow conduction with cellular/subcellular resolution. Third, the theoretical prediction of a transient increase in maximal upstroke velocities (dV/dt_{max}) of the propagated action potential during decreasing gap junctional coupling, was, with one exception, never verified experimentally. It was therefore the aim of the present study to: (1) determine minimal \( \theta \) during either a reduction of excitability or a reduction of gap junctional coupling, (2) investigate the characteristics of activation patterns on the microscopic level during slow conduction induced by either intervention, and (3) characterize changes in dV/dt_{max} during progressive gap junctional uncoupling. All of these issues were addressed by optically following impulse propagation at the multicellular and cellular level in patterned growth linear strands of cultured ventricular myocytes in which individual cells contributing to the propagation process could be identified. The findings show that partial gap junctional uncoupling can reduce \( \theta \) to a degree that could support microneurant excitation at pathlengths \( \leq 1 \) mm in rat ventricular tissue. Furthermore, during progressive uncoupling, cellular activation patterns change from being uniform to being highly discontinuous due to pronounced spatial differences in the degree of electrical coupling among the cells.

Materials and Methods

Patterned Growth Cell Cultures

Cell cultures from neonatal rat heart (Wistar) exhibiting predefined growth patterns were prepared according to previously published procedures. The dissociated ventricular myocytes were seeded at a density of \( 1.9 \times 10^5 \) cells/mm² on conditioned coverslips that caused the cells to grow according to predefined patterns. During the culturing period, the cells were incubated with M199 with Hanks’ balanced salt solution (Gibco) containing 5% neonatal calf serum (Animed, Allschwil, Switzerland), penicillin (20 000 U/L; Fakola), streptomycin (34 \( \mu \)mol/L; Fakola), vitamin B12 (15 \( \mu \)mol/L; Sigma), vitamin C (18 \( \mu \)mol/L; Sigma), bromodeoxyxuridine (100 \( \mu \)mol/L; Sigma), and epinephrine (10 \( \mu \)mol/L; Sigma). The cellular patterns consisted of linear cell strands (10 mm long: 50 to 300 \( \mu \)m wide). Whereas the narrow strands (4 to 6 cells wide) exhibited an anisotropic structure with elongated cells aligned with the main axis of the preparation, the cells in the center of the wide strands (\( \approx 20 \) cells wide) were aligned randomly, resulting in a preparation with a more isotropic structure. The strands were fabricated using a previously described photolithographic technique that was slightly modified to permit the coating of the regions supporting cell growth with collagen (human placenta type VI, Sigma; S.R. and R. Flückiger, unpublished data, 1998).

Optical Recording of Electrical Activation Patterns

Impulse propagation in the cell strands was followed optically using a fast voltage-sensitive dye. In short, the preparations were stained for 3 to 4 minutes with 1 mL of the superfusion solution containing 135 \( \mu \)mol/L of the voltage-sensitive dye di-8-ANEPPS (Molecular Probes). The dye was excited by a short-arc xenon lamp driven by a low-ripple power supply (Optiquip). The light was, after passing a shutter (VS25S21 MO, Vincent Assoc), short-pass filtered (570 EFS, Omega) and deflected toward the objective by means of a dichroic mirror (575 mm; Omega). Emitted fluorescence from the preparation was low-pass filtered (OG 590, Omega) and projected onto a 2-dimensional array of 379 optical fibers, which formed a hexagonal array. From the entire array, \( \leq 80 \) fibers were selected according to the shape of a given preparation and were connected to individual photodiodes. The resulting photocurrents were converted to voltages and amplified (typical overall gain of 2.5 \( \times \)10⁶ V/A; f = 1.6 kHz). The conditioned signals were simultaneously sampled at 20 kHz/channel by a computer-based data acquisition system (Pentium 133 MHz, Datagate) equipped with 2 40-channel analog-to-digital converters (PCI 20501C, 12-bit; Burr Brown). Experiments were performed with either a 20×, 0.75 NA objective (each detector monitored a circular area with a diameter of 50 \( \mu \)m corresponding to 4 to 10 cells contributing to the signal) or a 100×, 1.40 NA objective (circular tissue area with a diameter of 10 \( \mu \)m corresponding to \( \approx 1 \) to 4 cells contributing to the signal). With these objectives, activation could be assessed along the preparations for distances up to 1 mm (20×) and 200 \( \mu \)m (100×), respectively.

Experimental Protocol

After mounting the preparations in the experimental chamber, control superfusion was started (Hanks’ balanced salt solution, HBSS, containing (mmol/L) NaCl 137, KCl 5.4, CaCl₂ 1.3, MgSO₄ 0.8, NaHCO₃ 4.2, KH₂PO₄ 0.5, NaH₂PO₄ 0.3, and HEPES 10, which was titrated to pH 7.40 with NaOH). The preparations were stimulated with bipolar electrodes consisting of glass micropipettes (filing, HBSS with 1% agar) and a silver wire coiled around the shank of the electrode. The electrodes were attached to micromnipulators (DC-3K, Mahrþhaufer) and placed at a sufficient distance from the measurement site (\( \approx 1 \) mm) to (1) avoid electrotonically mediated stimulation artifacts to distort the signal of interest and (2) permit propagation to reach steady-state conditions at the site of the measurement. Rectangular impulses (duration, 1 ms; 2× threshold intensity) were delivered to the preparations at a basic cycle length of 500 ms by a stimulator (SD9, Grass Instruments) for at least 10 seconds before a given optical recording. All experiments were performed at a temperature of 36±0.4°C.

Experimental Solutions

Experimental superfusion solutions (HBSS) contained either an increased potassium concentration, tetrodotoxin (TTX; Calbiochem), palmitoleic acid (PA; Sigma) or 1-octanol (Merck). PA was brought into suspension by sonication of the superfusion solution in a bath sonicator (Ultrasonik, Ney). Octanol was dissolved by thorough stirring in a tightly capped flask.

Data Analysis

The raw data were analyzed by programming routines written in Interactive Data Language (IDL; Creaso). The data processing typically involved manual selection of the upstream portions of the action potential to omit any signal distortion due to motion artifacts occurring several milliseconds after the upstrokes. The data were digitally low-pass filtered by convolution with a 101-element finite impulse response filter (built-in function of IDL) at corner frequencies of 3.0 kHz (experiments with 100× objective), 1.5 kHz (experiments with 20× objective), and 0.5 kHz (high-[K⁺] experiments, with 20× objective). The signal amplitudes obtained under control conditions were set to 100%. Assuming an average action potential amplitude (APA) of 100 mV², the scaled values given as %APA translate directly into millivolts. Because the voltage-sensitive dye tended to internalize and bleach during repetitive illuminations, APAs obtained during repeated measurements at the same site were corrected in size under the assumption of a linear decay between the first (control) and the last (washout) measurement. This assumption was based on previously published data and on the absolute size of the decay in the present experiments, which was related linearly to the number of illuminations (\( \sim 16.7 \) %APA for 3 sequential illuminations, TTX, and PA experiments; \( \sim 32.2 \) %APA for 6 sequential illuminations, potassium experiments). Local activation times for each recording site were determined by averaging activation times obtained for 40% and 60% depolarization values in steps of 2% (t₅₀). From these values, both isochrones of activation and \( \theta \) (cm/s) were calculated. \( \theta \) was determined from the slope of a linear least-square fit of activation times recorded along the preparation.
Only longitudinal velocities were determined, because the structure of the strands (virtually 1-dimensional narrow strands and mostly isotropic wide strands) did not permit a meaningful separation of longitudinal from oblique and transverse propagation in their proper sense. To obtain a measure of the degree of uniformity of conduction, the standard deviation of the residuals of the linear regression was normalized to the mean activation delay, ie, to the time increment between neighboring recording sites determined from the fit (variation of activation delays [VAD], given as %).

Estimates of dV/dt max From Optical Recordings

Values for dV/dt max were calculated in relation to %APA and are given as %APA/ms (making the assumption of an average APA of 100 mV under control conditions, %APA/ms corresponds to V/s). Although this procedure was adequate for measurements of dV/dt max during uniform and relatively fast conduction at either optical magnification, it was inadequate during very slow and discontinuous conduction assessed at low spatial resolution because of the following reasons: (1) optical recordings produced a substantial underestimate of dV/dt max because many cells (up to 10 within the recording area of each detector) contributed in a staggered fashion to the recorded upstroke, thus artifactualy slowing it down; and (2) when motion artifacts were present, contractions originating from the first activated cells within a given recording area distorted the compound upstroke at ultra-slow VAs because the motion started before all cells within the recording area were activated. To eliminate these artifacts, only high-resolution measurements (1 to 4 cells contributing to the signal) were used in the quantitative assessment of the effect of electrical uncoupling on dV/dt max. Furthermore, only smoothly rising signals (ie, signals produced presumably by individual cells or by small clusters of virtually simultaneously activated cells) were included in the analysis, because it could not be decided unambiguously for the cases of action potential upstrokes displaying multiple phases (“notches”) whether these notches were due to electrotonic interactions or optical summation of delayed

![Figure 1. TTX-induced slow conduction in a narrow cell strand. The photograph depicts the preparation, and the circles indicate the areas imaged by individual photodetectors. The preparation was driven at 2 Hz (stimulation electrode positioned 1 mm above the field of view), and conduction was assessed during both control conditions (left) and superfusion with 22 μmol/L TTX (right). Signal amplitudes obtained during TTX superfusion were scaled to control values.](http://circres.ahajournals.org/doi/abs/10.1161/01.RES.81.10.783?journalCode=rcrse)

**TABLE 1. Effects of Sodium Current Block With 22 μmol/L TTX on Action Potential and Conduction Characteristics**

<table>
<thead>
<tr>
<th>Width, μm</th>
<th>n</th>
<th>Control</th>
<th>TTX</th>
<th>Wash</th>
<th>Control</th>
<th>TTX</th>
<th>Wash</th>
<th>Control</th>
<th>TTX</th>
<th>Wash</th>
<th>Control</th>
<th>TTX</th>
<th>Wash</th>
</tr>
</thead>
<tbody>
<tr>
<td>68±9</td>
<td>12</td>
<td>100±0</td>
<td>94.4±3.9*</td>
<td>100±0</td>
<td>96.2±4.8</td>
<td>22.1±3.7*</td>
<td>84.4±11.2*</td>
<td>38.1±4.4</td>
<td>13.3±2.0*</td>
<td>33.8±3.0*</td>
<td>29±12</td>
<td>47±19*</td>
<td>29±11</td>
</tr>
<tr>
<td>233±23†</td>
<td>13</td>
<td>100±0</td>
<td>86.0±5.6*†</td>
<td>100±0</td>
<td>99.8±3.3</td>
<td>16.3±2.0†</td>
<td>94.7±6.0†</td>
<td>37.0±3.0</td>
<td>10.0±2.0†</td>
<td>35.0±4.0*</td>
<td>52±9.0†</td>
<td>85±21†</td>
<td>56±7.0†</td>
</tr>
</tbody>
</table>

*P<0.005 vs control.
†P<0.005 narrow vs wide strands.
activation of electrically uncoupled cells. Finally, because only upstrokes produced by individual or simultaneously activated cells were included in the analysis, the delay in E-C coupling prevented motion artifacts to distort the action potential upstrokes.\textsuperscript{23}

Statistics

Values (mean±SD) were compared using the Student $t$ test (2-tailed, homo- or heteroscedastic where appropriate), and differences were considered significant at $P<0.005$.

Results

TTX-Induced Conduction Slowing

An experiment illustrating the contribution of $I_{Na}$ to impulse propagation in a patterned growth linear cell strand is shown in Figure 1. Under control conditions, $\theta$ in the 60-\textmu m-wide strand amounted to 43 cm/s, and the VAD was small (30\%), indicating uniform propagation. During superfusion with TTX (22 \textmu mol/L for 6 minutes), APAs were slightly reduced by 7.7\%, upstrokes remained smooth, and $dV/dt_{\text{max}}$ fell from 97.0 \%APA/ms to 18.8 \%APA/ms. Conduction velocity was reduced by $\sim$70\% to 13 cm/s, whereas VAD rose moderately to 53\%, indicating persistence of essentially uniform conduction. Because the concentrations of TTX used are known to induce a nearly complete block of $I_{Na}$,\textsuperscript{27} slow conduction in the presence of this drug was presumably carried exclusively by $I_{Ca}$. This conclusion was supported by a series of experiments in which local superfusion of the strands with TTX

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**TABLE 2. Effects of Increased [K$^+$]$_o$ on Action Potential and Conduction Parameters**

<table>
<thead>
<tr>
<th>[K$^+$]$_o$, mmol/L</th>
<th>n</th>
<th>Amplitude, %APA</th>
<th>Upstroke Velocity, %APA/ms</th>
<th>Conduction Velocity, cm/s</th>
<th>Variation of Activation, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.8, control</td>
<td>12</td>
<td>100±0</td>
<td>105.9±5.9</td>
<td>46.5±4.4</td>
<td>53±14</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>107.0±11.3</td>
<td>110.5±11.3</td>
<td>41.2±3.1</td>
<td>44±15</td>
</tr>
<tr>
<td>8.5</td>
<td>12</td>
<td>95.1±8.1</td>
<td>80.5±9.0*</td>
<td>41.6±5.9</td>
<td>61±21</td>
</tr>
<tr>
<td>14.8</td>
<td>12</td>
<td>82.9±10.6*</td>
<td>18.8±2.2*</td>
<td>16.3±2.8*</td>
<td>99±22*</td>
</tr>
<tr>
<td>30</td>
<td>12</td>
<td>70.2±9.2*</td>
<td>16.6±3.3*</td>
<td>14.9±3.4*</td>
<td>...</td>
</tr>
<tr>
<td>5.8, wash</td>
<td>12</td>
<td>100±0</td>
<td>103.9±5.2</td>
<td>46.8±6.1</td>
<td>60±38</td>
</tr>
<tr>
<td>14.8+22 \textmu mol/L TTX</td>
<td>6</td>
<td>87.4±7.5</td>
<td>18.4±2.1*</td>
<td>12.7±3.3*†</td>
<td>100±13*</td>
</tr>
</tbody>
</table>

Measured in narrow cell strands (width, 72±15 \textmu m; n=12).

*P<0.005 vs control.

†NS vs value at [K$^+$]$_o$=14.8.
(10\, \mu\text{mol/L}) and verapamil (10\, \mu\text{mol/L}) over lengths \geq 1\, \text{mm} consistently induced conduction blocks (S.R., unpublished data, 1997).

A compilation of the results obtained during block of I_{\text{Na}} with 22\, \mu\text{mol/L} TTX in both narrow (68 \pm 9\, \mu\text{m}; n=12) and wide cell strands (233 \pm 23\, \mu\text{m}; n=13) is shown in Table 1. Under control conditions, the only significant difference between the 2 types of strands concerned VAD (narrow, 29\%\pm 12\%; wide, 52\%\pm 9\%). This difference seemed to be preparation-specific, because it did not reoccur during subsequent experiments (Table 3). During block of I_{\text{Na}}, all parameters changed in a similar way in both types of preparations. Conduction velocity was reduced from 38\%\pm 4 to 13\%\pm 2 m/s in narrow strands and from 37\%\pm 3 to 10\%\pm 2 cm/s in wide strands. TTX induced a slightly larger depression of both %APA and dV/dt_{\text{max}} in wide strands. During slow conduction, VAD increased from 29\% to 47\% in narrow strands and from 52\% to 85\% in wide strands. Thus, slight nonuniformities of conduction present under control conditions were enhanced by the same degree (60\%) in both types of preparations during superfusion with TTX. Washout failed to completely restore dV/dt_{\text{max}}.

Figure 3. PA-induced very slow conduction in a narrow cell strand. The photograph depicts the preparation, and the circles indicate the areas imaged by individual photodetectors. The preparation was driven at 2 Hz from above, and conduction was assessed during both control conditions (left) and washout after a previous superfusion with 20\, \mu\text{mol/L} PA (right; for experimental protocol, see Materials and Methods). Signal amplitudes obtained during washout were scaled to control values. Arrows indicate multiple notches in the upstroke of one of the signals (for explanation, see Results). *Signals showed no clear plateau because of the presence of motion artifacts and were clipped when reaching 100\%APA, as determined during the control recording.
Figure 4. Ultra-slow conduction in a wide cell strand during partial gap junctional uncoupling with PA. A. Control conditions: phase-contrast image of the preparation. ● indicates centers of the regions imaged by individual photodetectors. The disk diameter is proportional to the dV/dt max (scale to left of photograph). The cell strand was stimulated on the left at 2 Hz, and the color-coded band with overlaid isochrone lines of activation (isochrone interval = 100 μs) illustrates the spatial characteristics of activation along the preparation. The panels below the photograph depict action potential upstrokes (black) and their time derivatives (dV/dt; red) recorded from the 3 sites in the preparation marked by green circles. B. Ultra-slow conduction during partial gap junctional uncoupling as measured during recovery from a previous superfusion with 20 μmol/L PA. Layout as in (A) (note increase of isochrone interval by a factor of 100).
and $\theta$ to control values, which probably was due to either noncomplete washout of the drug or to the onset of phototoxic effects induced by the repetitive illuminations.

**Slow Conduction Induced by Elevated Extracellular Potassium**

Whereas the experiments with TTX illustrated the characteristics of slow propagation during suppression of $I_{Na}$ in normally polarized tissue, slow propagation in the setting of acute ischemia occurs in depolarized tissue. To characterize conduction under this condition, narrow strands (width, 81±15 $\mu$m; n=12) were exposed to increasing concentrations of extracellular potassium ($[K^+]_o$, in mmol/L: 2.0, 5.8=control, 8.5, 14.8, 30). As shown in Figure 2A and Table 2, APAs (%APA) were inversely related to $[K^+]_o$, showing a log-linear dependence in the range of $[K^+]_o$,=5.8 mmol/L to $[K^+]_o$,=30 mmol/L. At the same time, $dV/dt_{max}$ showed a sigmoidal behavior with a maximal decline between 5.8 and 14.8 mmol/L ($[K^+]_o$, (Figure 2B). Conduction velocity (Figure 2C and Table 2) was fastest at $[K^+]_o$,=5.8 mmol/L and decreased significantly at both lower and higher concentrations of extracellular potassium. Increasing $[K^+]_o$, from 14.8 mmol/L to 30 mmol/L affected $\theta$ only minimally. Also, addition of 22 µmol/L TTX to the superfusion solution containing 14.8 mmol/L ($[K^+]_o$), led to no further significant reduction in $\theta$ (Table 2), suggesting that the highest concentrations of $[K^+]_o$, used (14.8 and 30 mmol/L) induced full block of $I_{Na}$ and that conduction was primarily supported by $I_{Ca}$. With increasing $[K^+]_o$, $\theta$ first increased, whereas $dV/dt_{max}$ decreased (2→5.8 mmol/L ($[K^+]_o$), “supernormal conduction”). Then, both parameters decreased, reaching steady values for both $[K^+]_o$,=14.8 mmol/L and $[K^+]_o$,=30 mmol/L. (Figure 2D).

During the increase in $[K^+]_o$, VAD increased moderately from 44% ($[K^+]_o$,=2.0 mmol/L) to 61% ($[K^+]_o$,=8.5 mmol/L) before rising significantly above control values at $[K^+]_o$,=14.8 mmol/L (99%; Table 2). Because signal-to-noise ratios at $[K^+]_o$,=30 mmol/L were small because of the reduction in signal amplitudes, VAD values could not be calculated for this condition and are therefore omitted in Table 2.

To determine the maximal concentration of extracellular potassium still permitting successful conduction, $[K^+]_o$, was raised to 50 and 60 mmol/L in a series of additional experiments (n=5). Whereas at $[K^+]_o$,=50 mmol/L, conduction never failed, complete block was observed in all experiments at $[K^+]_o$,=60 mmol/L.

**Very Slow Conduction During Partial Gap Junctional Uncoupling**

To assess the extent of maximal conduction slowing induced by partial gap junctional uncoupling, linear cell strands were exposed to 20 µmol/L PA. This fatty acid has been shown before to be a potent gap junctional uncoupler that does not affect action potential shapes in cultured rat cardiomyocytes, thus having presumably no major effects on membrane currents. Experiments were performed with both narrow strands (79±15 $\mu$m wide; n=12) and wide strands (268±14 $\mu$m wide; n=10), and impulse propagation was assessed at low spatial resolution (50 $\mu$m), permitting the assessment of the characteristics of conduction over a relatively long segment of the preparation (1 mm) at the expense of being unable to reliably calculate $dV/dt_{max}$ during uncoupling (see Materials and Methods). After a control recording, the preparations were superfused uniformly with PA until complete uncoupling occurred as judged by cessation of contractile activity during continued stimulation (3±1 minutes; n=22). Thereafter, washout of PA was started, and a recording was initiated when the preparations again showed 1:1 conduction (5±1 minutes; n=22). The last recording (washout control) was obtained >15 minutes after switching to the control solution.

An example for very slow conduction induced in a narrow cell strand by partial gap junctional uncoupling with PA is illustrated in Figure 3. Under control conditions, conduction was fast and uniform ($\theta$=47.2 cm/s), and the upstrokes of the propagated action potential were smooth. During partial uncoupling, $\theta$ fell by ~98% to 0.9 cm/s, and upstrokes became slow, some of them displaying multiple notches. Most of this decrease in upstroke velocity was artifactual, because during these low resolution measurements, each recording site integrated upstrokes from many cells activated with large delays, thus producing a slowly rising compound upstroke (see Materials and Methods). Furthermore, a clear action potential plateau was missing in some traces, because the compound upstroke was smoothly fused with a positive going motion artifact.

Activation in wide cell strands during partial uncoupling typically followed a meandering pathway as shown in the example depicted in Figure 4. Under control conditions (Figure 4A), activation proceeded along the 230-µm-wide preparation in a uniform manner, as indicated by the mostly parallel and evenly spaced isochrones and by the linear increase of activation times along the preparation (Figure 4C, blue trace; $\theta$=36.7 cm/s). As shown for 3 selected recording sites, action potential upstrokes under control conditions were smooth with $dV/dt_{max}$ >100%APA/ms. In contrast, during partial gap junctional uncoupling (Figure 4B), conduction was not only drastically slowed by >2 orders of magnitude to 0.31 cm/s (note 100× increase in isochrone intervals), but activation of the preparation became highly tortuous because of the presence of patches of completely uncoupled groups of cells (hatched regions), which diverted the approaching activation wavefront. This type of conduction was accompanied by the occurrence of action potential upstrokes displaying any of the following characteristics (see selected signals in lower part of Figure 4B): (1) absence of gross distortions...
in the action potential upstroke (leftmost signal); (2) lack of signals in regions of the preparation, which were completely uncoupled (middle signal); or (3) signals displaying multiple notches in their upstrokes (rightmost signal), indicating sequential activation of cells or groups of cells with relatively large delays. For the plot of activation times along a straight line of the preparation (Figure 4C, red trace), the presence of meandering activation resulted in local regions displaying slow conduction (1.0 cm/s ≤ θ ≤ 8.4 cm/s; n = 4), ultra-slow conduction (0.09 cm/s ≤ θ < 1 cm/s; n = 10), and conduction in reversed direction (−4.7 cm/s ≤ θ ≤−0.2 cm/s; n = 4).

The results of all PA experiments in both narrow and wide cell strands are summarized in Table 3. Under control conditions, θ tended to be slower in wide strands than in narrow strands, which most likely is due to differences in the cellular architecture: because the cells in the wide strands were not aligned in parallel but were mostly oriented at random, the number of cell-to-cell appositions per unit length was increased, thus causing slower steady-state θ. After superfusion with PA, θ was drastically reduced from 47 to 1 cm/s (narrow strands) and from 42 to 0.5 cm/s (wide strands). The minimal velocity observed in wide strands was 0.25 cm/s. This reduction was accompanied by an increase in VAD in narrow strands from 37% to 104% and in wide strands from 44% to 161%. As for the TTX experiments, washout failed to completely restore θ to control values, which most probably was due to either a noncomplete washout of the drug or to the onset of phototoxic effects induced by repetitive illuminations.

### High-Resolution Determinations of Activation Characteristics During Partial Uncoupling

The experiments discussed above established the characteristics of slow conduction during gap junctional uncoupling at the multicellular level (spatial resolution, 50 μm) over relatively long segments of the preparations (800 to 1000 μm). Experiments aimed at resolving cell-to-cell propagation were performed with a spatial resolution of 10 μm. During these experiments performed with objectives having high numerical apertures (100×; 1.4 NA), light intensities at the level of the preparations were roughly 90× higher than during the low magnification experiments. This resulted in the development of phototoxic effects such as a decrease of dV/dt_{max} and a progressive conduction slowing even after a single exposure. Therefore, control experiments and interventions had to be carried out at different locations of the preparations. An example of a control recording obtained at high spatial resolution in a narrow cell strand (55 μm wide) is shown in Figure 5. The preparation was stimulated at the left and exhibited an average θ of 43 cm/s. As indicated by the mostly parallel and evenly spaced isochrones (Figure 5A) and by the smoothly rising upstrokes (Figure 5B), conduction was uniform at the cellular level, showing no major interferences from the cell borders.

The microscopic activation characteristics changed dramatically during gap junctional uncoupling as illustrated in Figure 6 by the results of an experiment obtained in a different preparation. A narrow cell strand (55 μm wide, Figure 6A) was completely uncoupled with PA and, immediately after reestablishment of conduction during washout, activation was assessed at the sites indicated by yellow circles. As can be seen from the temporal patterning of the signals (Figure 6B), conduction was not only highly slowed (overall delay, ≈16 ms; θ = 1.1 cm/s), but activation advanced stepwise along the preparation as indicated by the clustering of the action potential upstrokes. In contrast to the experiments performed at low spatial resolution (Figure 3), action potential upstrokes were faster because detectors received signals from ≤4 cells, thus reducing the problem of optical integration (see Materials and Methods). The statistics of activation were determined for each individual cluster of action potential upstrokes based on the point in time of occurrence of dV/dt_{max} (t_{Vmax}) or the point in time of occurrence of 50% depolarization (t_{50}). The results of both modes of calculation were in close agreement and yielded activation delays between consecutive clusters in the ranges of 0.54 to 4.52 ms (mean, 2.69 ms; t_{50}) and 0.41 to 4.45 ms (mean, 2.53 ms; t_{50}). In contrast to these large intercluster delays, the standard deviation among activation times within the clusters was very small, ranging from 0 to 350 μs (mean, 130 μs; t_{50}) and 80 to 450 μs (mean, 190 μs; t_{50}), thus indicating the presence of highly discontinuous conduction. The spatial origin of the clustered signals is illustrated in Figure 6C, which shows the projection of all recording sites onto a schematic drawing of the preparation with highlighted borders of individual cells. Recording sites are color-coded according to the colors used in Figure 6B. White discs correspond to sites showing no action potential, ie, conduction block. Detector sites reporting signals with ill-defined upstrokes or notched upstrokes (shown as dashed lines in Figure 6B) are cross-hatched. This figure illustrates that clustered activity originated from small patches of the preparation consisting of 1 to 3 cells, in which the patches were activated sequentially with variable delays. As indicated qualitatively by the dashed arrows, activation invaded the preparation in a tortuous manner because of the presence of a central obstacle consisting of a single cell (cross hatched outline). This cell, which was still completely uncoupled at

### Table 3. Effects of Gap Junctional Uncoupling on Action Potential and Conduction Characteristics

<table>
<thead>
<tr>
<th>Width, μm</th>
<th>n</th>
<th>Control</th>
<th>PA</th>
<th>Wash</th>
<th>Control</th>
<th>PA</th>
<th>Wash</th>
<th>Control</th>
<th>PA</th>
<th>Wash</th>
</tr>
</thead>
<tbody>
<tr>
<td>79±15</td>
<td>12</td>
<td>100±0</td>
<td>115±20</td>
<td>100±0</td>
<td>47.05±1.95</td>
<td>0.99±0.80*</td>
<td>38.41±2.60*</td>
<td>37±10</td>
<td>104±29*</td>
<td>36±9</td>
</tr>
<tr>
<td>268±14†</td>
<td>10</td>
<td>100±0</td>
<td>112±14</td>
<td>100±0</td>
<td>41.83±1.77†</td>
<td>0.46±0.19*</td>
<td>33.96±1.90*</td>
<td>44±9</td>
<td>161±29†</td>
<td>59±36</td>
</tr>
</tbody>
</table>

*P<0.005 vs control.
†P<0.005, narrow vs wide strands.
the time of the measurement, forced the activation to take a turn, resulting in a region of the preparation exhibiting backward propagation. Microscopic conduction patterns exhibiting characteristics similar to the example shown in Figure 6 were observed in an additional 5 experiments with narrow strands in which $u$ ranged from 0.4 to 1.1 cm/s during partial gap junctional uncoupling. Thus, in addition to mean-dering impulse propagation observed in wide cell strands at a multicellular level, these high-resolution mappings revealed that activation was, to a certain extent, meandering at the microscopic level in narrow strands, as well. Furthermore, the measurements showed that impulse propagation during gap junctional uncoupling is highly discontinuous at the cellular scale, exhibiting “saltatory” conduction from microscopic tissue patches to the next.

Relationship Between $\theta$ and $dV/dt_{\text{max}}$ During Progressive Uncoupling

Previous computer simulations have consistently predicted that the progressive decrease of $\theta$ during progressive gap junctional uncoupling is, at the cellular level, accompanied by an initial increase and then, during very slow propagation, by a substantial decrease in $dV/dt_{\text{max}}$. With the exception of 1 report, in which 1-octanol was used as uncoupling agent, experimental studies so far have failed to show such a transient increase in $dV/dt_{\text{max}}$. Because such experiments had never been performed with PA before, it was of interest to investigate the change of $dV/dt_{\text{max}}$ during progressive uncoupling induced by either PA or 1-octanol. The results of these experiments performed at high spatial resolution (10 $\mu$m) are summarized in Figure 7. During progressive uncoupling with 20 $\mu$mol/L PA (Figure 7A), the decrease in $\theta$ was accompanied by a moderate decrease in $dV/dt_{\text{max}}$, leveling off at $\theta<$ 20 cm/s at a value of $\approx$ 130 %APA/ms. In contrast, progressive gap junctional uncoupling induced by 1-octanol (300 to 600 $\mu$mol/L; Figure 7B) was characterized by a steady decrease of $dV/dt_{\text{max}}$ to $\approx$ 40 %APA/ms before occurrence of conduction blocks. As shown in Figure 7C, differences in the degree of depression of $dV/dt_{\text{max}}$ between the 2 uncoupling agents became significant at $\theta>$ 30 cm/s. However, even though PA depressed $dV/dt_{\text{max}}$ to a significantly smaller extent than 1-octanol, it failed to show the transient increase in $dV/dt_{\text{max}}$ predicted by computer simulation studies.

Discussion

Slowing of Conduction During a Reduction of Excitability

It has been shown in many studies that a progressive increase of $[K^+]_o$ is accompanied, first, by an increase of $\theta$ (“supernormal conduction”; maximal $\theta$ reported at $[K^+]_o$ of (in mmol/L) 4.0, 6.0, 7.4, 9.0, and 10.8) and then by a progressive decrease up to the point of conduction block (blocks reported at $[K^+]_o$ of 14 mmol/L and 16 mmol/L). In these studies, slowest $\theta$s measured before occurrence of
Figure 6. Characteristics of microscopic impulse propagation during ultra-slow conduction induced by gap junctional uncoupling. A, Phase-contrast image of the preparation (narrow cell strand, width, 55 µm) with circles indicating the spatial arrangement of the photodetectors. B, Plot of action potential upstrokes recorded simultaneously by all detectors during activation of the preparation from the left. Temporally coinciding upstrokes are color-coded and the respective activation times determined from either the point in time of occurrence of dV/dt_{max} (t_{V_{max}}) or the point in time of occurrence of 50% depolarization (t_{50}) are shown below the graph. Dashed lines refer to signals that could not be attributed unequivocally to any set of upstrokes (see Results). C, Local activation map. The recording sites producing the upstrokes shown in (B) are color-coded accordingly and superimposed on a schematic drawing of the preparation showing the cell borders. At the sites of white discs, no changes in transmembrane voltage were recorded (electrically uncoupled cell with hatched outline). The cross-hatched discs indicate the locations of signals for which it was not possible to assign unambiguous activation times because of the presence of notched upstrokes (dashed signals in [B]). Arrows indicate qualitatively the direction of activation that advanced in a stepwise fashion along the preparation (overall v ~1.1 cm/s).
blocks and in the absence of adrenergic stimulation were, with the exception of findings by Cranefield and colleagues, in the range of 20 to 40 cm/s.2–5 Whereas the preparations used in the present study exhibited similar responses to moderate increases of $[K^+]_o$ (supernormal conduction at $[K^+]_o=5.8$ mmol/L), they differed in respect to $[K^+]_o$ needed to induce conduction block (60 mmol/L) and in respect to minimal $\theta$s obtained before block (15 cm/s at $[K^+]_o=30$ mmol/L). Furthermore, TTX never induced conduction blocks, whereas such blocks have been reported to occur systematically in intact cardiac tissue.3,12 Because the concentrations of TTX used are known to induce a nearly complete block of $I_{Na}$, slow conduction in the presence of this drug was presumably carried exclusively by $I_{Ca}$. This was also the case for $[K^+]_o=14.8$ mmol/L as evidenced by the finding that $22 \mu$mol/L TTX added to $[K^+]_o=14.8$ mmol/L had no significant effect on APAs, $dV/dt_{max}$, or $\theta$. Thus, $I_{Ca}$ in the cultured cell strands was obviously large enough to support slow conduction, even in the presence of highly elevated $[K^+]_o$, and in the absence of adrenergic stimulation.4

Although peak $I_{Ca}$ was described to be comparable in size in cultured and adult rat ventricular myocytes,33 it has been reported that both the presence of serum33,34 or norepinephrine35 during the culturing period increases the density of L-type calcium channels. Thus, it is feasible that the Ca$^{2+}$ current density in the preparations used in the present experiments was slightly enhanced, thus resulting in stable conduction during both large increases of $[K^+]_o$ and in the presence of TTX.

Irrespective of the use of TTX or elevated $[K^+]_o$, the study showed that activation at the microscopic level remained essentially uniform during conduction slowing, shown by the low VAD values observed during either interventions. This suggests that (1) electrical coupling among the cells remained undisturbed, and (2) the density of $I_{Ca}$ was uniform among the cells, thus preventing the occurrence of variable local activation delays based on current-to-load mismatches.

### Slowing of Conduction During Gap Junctional Uncoupling

It has been shown by computer simulations that a reduction of gap junctional coupling reduces $\theta$ to a much greater extent than a reduction of $I_{Na}$,8,14,16,18 thus suggesting a higher safety factor for conduction during uncoupling. Qualitatively, this prediction compares well with published experimental data obtained in ventricular tissue preparations during longitudinal propagation, as critical K-depolarizations, ie, depolarizations just below the point of occurrence of conduction blocks, generally reduced $\theta$ to a lesser extent (40% to 60%3–5) than critical gap junctional uncoupling (60% to 85%6,7,9). Quantitatively, however, the simulations suggested a substantially higher maximal reduction of $\theta$ during uncoupling ($\approx95%8,14,18$). In close agreement with these findings, reductions of $\theta$ achieved in the present study during critical uncoupling were 98% and 99% in narrow and wide strands, respectively. The largest reduction of $\theta$ (43.5 to 0.25 cm/s; 174-fold reduction) was actually nearly identical to that reported by the most recent simulation studies (56 to 0.26 cm/s; 200-fold reduction).14 The uncoupling-induced reduction of $\theta$ was accompanied by typical changes of the activation pattern at the multicellular level; whereas, in narrow cell strands, activation remained largely uniform, the activation wavefront in wide strands was frequently changing directions, resulting in a meandering pattern of activation that is likely to explain the larger slowing of conduction observed in wide versus narrow strands (0.5 versus 1.0 cm/s). These meanders were induced by patches of completely uncoupled

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**Figure 7.** Relationship between $dV/dt_{max}$ and $\theta$ during a progressive increase in gap junctional resistance. A, Data obtained during gap junctional uncoupling with 20 $\mu$mol/L PA. Each circle represents the average of $dV/dt_{max}$ values obtained from a single row of detectors at high spatial resolution (10 $\mu$m; $5\pm n=18$ for each data point). B, Same as (A) for 1-octanol (300 to 600 $\mu$mol/L). C, Comparison of the results shown in (A) and (B): $dV/dt_{max}$ values in bins of $\theta$ of 10 cm/s were pooled and are shown (mean$\pm$SD) both for PA (white columns) and 1-octanol (black columns). *Significant differences between PA and 1-octanol.
cells that forced the activation wavefronts to take turns, thus resulting in an apparent slowing of macroscopic $\theta$ reminiscent of “zig-zag” activation in infarcted\textsuperscript{11} and nonuniform anisotropic tissue in which, during premature stimulation, this type of activation caused a mean transverse $\theta$ of 4.2 cm/s.$^{12}$

Interestingly, when observed with cellular/subcellular resolution, activation was meandering in narrow cell strands, as well. The high-resolution measurements showed that propagation was sustained by sequentially activated tissue patches, each of which consisted of a few cells being activated simultaneously. At the same time, interspersed islands of completely uncoupled cells forced the activation wavefront to take turns, resulting in microscopically meandering activation. Thus, even though the preparations (1) displayed a uniform cellular architecture and (2) were subjected evenly to PA, conduction was not uniformly slowed, ie, the smallest “functional unit” consisted rather of small groups of cells than of individual cells. This finding most likely is explained by nonuniformities in the density of gap junctions in these cultures,$^{20}$ resulting in a spatially variable degree of intercellular coupling. Following the line of reasoning of a previous theoretical study,$^{15}$ such spatial variability (groups of well-coupled cells joined by large resistors) would result, at any given overall resistance, in a substantially slower $\theta$ than if intercellular resistances were to be distributed uniformly, thus adding to the explanation of ultra-slow conduction observed in this study.

The finding that gap junctional uncoupling reduced $\theta$ to a substantially higher degree than what previously has been found in intact cardiac preparations\textsuperscript{6,7,9} might have several reasons. First, both heptanol and octanol used in some of the above-mentioned studies impair nonjunctional membrane currents at the concentrations used for uncoupling the preparations.$^{36}$ In contrast, it has been reported that PA exerts no effects on action potential shapes of cultured neonatal rat heart cells when used at uncoupling concentrations.$^{28}$ Thus, $I_N$ and $I_{Ca}$ presumably were not impaired to a major extent in the present study, which could partly explain the greater resistance of the preparations to early conduction failure. Second, the possibility of an increase of $I_{Ca}$ in the cultured cells (see above) would have sustained conduction because of the increased availability of depolarizing current. Third, it can be envisaged that the access of uncouplers to intact tissue\textsuperscript{6,7,9} is less uniform than the access to the cell monolayers. Hence, uncoupling in intact tissue might have been less homogeneous, resulting in local current-to-load mismatches precipitating conduction blocks well ahead of blocks expected on the basis of uniform uncoupling.$^{15}$ Finally, because cultured myocytes are coupled all around their circumference to their neighboring cells\textsuperscript{29} as opposed to the predominant end-to-end coupling in adult intact tissue, it is feasible that the value of minimal $\theta$ encountered during uncoupling might differ to a certain extent from the situation in intact tissue.

Effects of Uncoupling on $dV/dt_{\text{max}}$

Computer simulations consistently have shown that the progressive decrease of $\theta$ during uncoupling is accompanied by a transient increase of $dV/dt_{\text{max}}$ due to the discrete cellular architecture of cardiac tissue.$^{6,8,17}$ With the exception of 1 study describing such a transient increase of $dV/dt_{\text{max}}$ during a 1-octanol–induced reduction of $\theta$ in guinea pig papillary muscle,$^{6}$ experiments performed with octanol, heptanol, or isoflurane produced a monotonic decrease of $dV/dt_{\text{max}}$ with increasing uncoupling.$^{8,9}$ In the present study, this was not different, as $dV/dt_{\text{max}}$ during PA-induced uncoupling showed a monotonic decrease. However, the maximal extent of this decrease was moderate, because $dV/dt_{\text{max}}$ was reduced by <35% as opposed to a reduction by 85% in the presence of 1-octanol. This large decrease of $dV/dt_{\text{max}}$ induced by 1-octanol is most likely explained by previous findings that describe a substantial suppression of $I_{Na}$\textsuperscript{36} and $I_{Ca}$\textsuperscript{37} by n-alkanols. Thus, it remains unexplained why $dV/dt_{\text{max}}$ did not show the simulation-predicted transient increase during progressive uncoupling even though, in terms of minimal $\theta$, the findings of the present study were in close agreement with the most recent simulation study\textsuperscript{14} and even though PA presumably had no major impact on nonjunctional membrane currents.$^{28}$

Minimal Pathlengths Supporting Reentrant Excitation

In anatomically defined reentry, the minimal length of the reentrant pathway has to be larger than the wavelength of excitation, ie, the product of $\theta$ and refractory period. In the present study, minimal pathlengths of narrow cell strands were 1 mm (1.0 cm/s×100 ms). This length represents a reliable estimate for cultured cell strands, because (1) activation had little freedom to take any substantially longer path than the one dictated by the narrow strand itself, and (2) ultra-slow propagation was not occurring locally but was observed consistently over the whole mapped distance of 800 to 1000 $\mu$m, ie, over the entire length necessary to support reentrant excitation. However, when extrapolating this value to intact tissue, a number of caveats apply. First, the restriction of the extracellular space will introduce an additional resistance to local current flow that can be expected to result in very low $\theta$ earlier in the process of acute uncoupling. Second, the cultured neonatal ventricular myocytes are $\approx$30% shorter than adult rat ventricular myocytes.$^{35}$ This implies an increased spatial frequency of cell-to-cell appositions and thus a decrease in the discontinuity of the resistive network. Based on previous computer simulations,$^{13}$ such a decrease would be expected to result, at a given stage of uncoupling, in slightly higher $\theta$s and, consequently, somewhat longer pathlengths in cultured than in adult cells. Third, gap junctions in the cultured preparations and, similarly, in neonatal and in peri-infarction tissue,\textsuperscript{38} are found around the cell circumference, whereas, in adult myocardium, gap junctions are located predominantly at the cell ends. In adult myocardium, therefore, acute cell-to-cell uncoupling is expected to affect conduction more prominently in the transverse direction, resulting in longitudinal dissociation of propagation before the occurrence of block. Fourth, tissue anisotropy, which was present only to a limited extent in the preparations used in the present study, is well-known to play an important role in the mechanism of arrhythmias. In seminal work, anisotropic reentry has been described in relatively large areas in peri-infarction tissue\textsuperscript{39}...
and in small volumes of atrial trabeculae. In the case of reentry in very small tissue areas, it was suggested that the sparse and irregular lateral coupling due to interspersed connective tissue sheets (so-called nonuniform reentry) forms the structural basis for reentry observed during premature stimulation. Although it is not yet possible to track and measure the length of the reentrant pathway with cellular resolution in such miniature 3-dimensional tissue volumes, $\theta_s$ as low as 2.5 cm/s have been observed in transverse direction to the main fiber axis in these experiments in the absence of acute uncoupling. Given that acute uncoupling reduced $\theta$ by yet another order of magnitude in the present study (0.25 cm/s), it remains to be shown whether the combination of acute uncoupling and marked nonuniform anisotropy will reduce the minimal pathlength even further.

Based on the coarse estimate of a minimal pathlength of 1 mm, it is possible to speculate about the minimal area of cardiac tissue that may host a reentrant pathway. A simple circular pathway would have a diameter of $\approx 500 \mu m$ (320 $\mu m$ plus twice the strand width) and cover a surface of 0.2 mm$^2$ (maximal area requirement). Any deviation from the circular shape by folding of the linear structure would be expected to decrease the area further. How do these estimates translate to the situation in vivo? Spach and colleagues observed reentrant excitation to follow a roughly rectangular path, circumscribing the smallest-ever–reported area harboring reexcitation (1.6 mm$^2$). The calculated pathlength in this case was $\approx 6$ mm. However, as stated by the authors, the real pathlength was probably longer because of the complex histology of the tissue consisting of small groups of cells running in parallel and exhibiting sparse side-to-side coupling. Because this result was obtained in the absence of pharmacological uncoupling, one might speculate that the combination of nonuniform anisotropic tissue and acute uncoupling could result in even smaller areas giving rise to reentrant excitation. This question might possibly be tackled by designing a cell pattern exhibiting the salient features of nonuniform anisotropic tissue, ie, a 2-dimensional pattern consisting of parallel running narrow strands of cells like the ones used in the present study, which would infrequently be coupled side-to-side. Such a structure would permit the establishment of the characteristics of reentrant excitation with cellular resolution in both the absence and presence of acute uncoupling in discontinuous anisotropic media.

In summary, it was shown that $\theta$ in linear cell strands of cardiac tissue is reduced to a far larger extent by partial gap junctional uncoupling than by a reduction of excitability. This reflects an increased margin of safety of propagation during electrical uncoupling. Whereas primarily $I_{Ca}$- supported conduction during suppression of $I_{Na}$ remained largely uniform, it became highly discontinuous during partial uncoupling. Ultra-slow conduction during critical uncoupling was characterized by activation advancing stepwise and with variable delays from small groups of cells to the next groups. In these groups, $dV/dt_{max}$ was reduced only slightly compared with controls. Minimal $\theta_s$ measured over $\approx 1$ mm–long segments were as low as a few millimeters per second, thus emphasizing the feasibility of micro-reentrant excitation to occur in tissue areas measuring considerably <1 mm$^2$.

Acknowledgment

This work was supported by the Swiss National Science Foundation. We would like to thank Mrs. R. Flückiger Labrada for the cell culture work.

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Slow Conduction in Cardiac Tissue, I: Effects of a Reduction of Excitability Versus a Reduction of Electrical Coupling on Microconduction
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Circ Res. 1998;83:781-794
doi: 10.1161/01.RES.83.8.781

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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