HIGHLIGHTED TOPIC | Biomechanics and Mechanotransduction in Cells and Tissues

Mechanoelectrical excitation by fluid jets in monolayers of cultured cardiac myocytes

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Kong, Chae-Ryon, Nenad Bursac, and Leslie Tung. Mechano-electrical excitation by fluid jets in monolayers of cultured cardiac myocytes. J Appl Physiol 98: 2328–2336, 2005. First published February 24, 2005; doi:10.1152/japplphysiol.01084.2004.—Although the prevailing view of mechanoelectric feedback (MEF) in the heart is in terms of longitudinal cell stretch, other mechanical forces are considerable during the cardiac cycle, including intramyocardial pressure and shear stress. Their contribution to MEF is largely unknown. In this study, mechanical stimuli in the form of localized fluid jet pulses were applied to neonatal rat ventricular cells cultured as confluent monolayers. Such pulses result in pressure and shear stresses (but not longitudinal stretch) in the monolayer at the point of impingement. The goal was to determine whether these mechanical stimuli can trigger excitation, initiate a propagated wave, and induce reentry. Cells were stained with the voltage-sensitive dye RH237, and multi-site optical mapping was used to record the spread of electrical activity in isotropic and anisotropic monolayers. Pulses (10 ms) with velocities ranging from 0.3 to 1.8 m/s were applied from a 0.4-mm diameter nozzle located 1 mm above the cell monolayer. Fluid jet pulses resulted in circular wavefronts that propagated radially from the stimulus site. The likelihood for mechanical stimulation was quantified as an average stimulus success rate (ASSR). ASSR increased with jet amplitude and time waited between stimuli and decreased with the application of gadolinium and streptomycin, blockers of stretch-activated channels, but not with nifedipine, a blocker of the L-type Ca channel. Absence of cellular injury was confirmed by smooth propagation maps and propidium iodide stains. In rare instances, the mechanical pulse resulted in the induction of reentrant activity. We conclude that mechanical stimuli other than stretch can evoke action potentials, propagated activity, and reentrant arrhythmia in two-dimensional sheets of cardiac cells.

mechano-electrical coupling; ventricular arrhythmias; optical mapping; cell culture; neonatal rat

Numerous studies have shown that mechanical stretch or load applied to a cardiac tissue can induce significant electrophysiological effects via the process termed “mechano-electric feedback” (MEF). Mechanically triggered action potentials have been observed with sudden stretch of the ventricles (20), stretch of the isolated ventricular free wall (17), or mechanical stimulation of single cardiac myocytes (13, 28, 42). Mechanical stretch also changes action potential duration (42, 48, 51), excitability (45), and expression of gap junctional channels (52). The electrophysiological effects have been attributed mainly to the activity of stretch-activated ion channels (SACs) (26). Pharmacological agents that inhibit SAC activity include streptomycin (21), gadolinium (Gd3+) (26), and the tarantula toxin GsMTx-4 (40).

The mechanically induced premature ventricular beats mentioned above can be proarrhythmic. As a dramatic example, commotio cordis is the phenomenon of a blunt chest trauma causing cardiac arrest without apparent cellular damage. In a swine model of commotio cordis, ventricular fibrillation can be induced with a projectile impact that occurs over the heart within the vulnerable period of the electrocardiogram (38). Pulsatile increases in diastolic ventricular volume result in premature extrasystolic beats (19, 22), and the pressure of a cardiac catheter against the ventricular wall can initiate extrasystoles (32, 43).

The prevailing view of MEF is in terms of tissue stretch, as is explicitly clear in computer models of MEF that encode the mechanical input as cell length or sarcomere length (31, 50). On the other hand, intramyocardial pressure/compression and shear stress/strain are also prevalent in tissue and play substantial roles in the mechanics of the heart. Intramyocardial pressure is important in governing coronary blood flow (2, 24), whereas shear stress/strain is a result of the laminar sheet structure of the heart (12) and is implicated with ventricular torsion and diastolic recoil (3, 34). Recently, it has been shown that compression and shear elicit different mechanosensitive responses in membrane ion currents when applied to single cardiomyocytes in the absence of cellular length changes (28).

Thus, although pressure and shear stress constitute potent mechanical forces with physiological consequences, their contribution to MEF is largely unknown. In this study we tested whether pressure and shear stress, applied via a fluid jet pulse, can 1) trigger excitation, 2) initiate a propagated wave, and 3) induce reentry in cardiac cells in the absence of longitudinal cell stretch. Multisite optical mapping was used to record the mechano-electrical responses in cultured monolayers of neonatal rat ventricular myocytes. Part of this work was reported previously in abstract form (33).

MATERIALS AND METHODS

Cell culture. All studies were performed in accordance with the American Physiological Society’s Guiding Principles in the Care and Use of Animals, and the animal protocol was approved by the Johns Hopkins Animal Care and Use Committee. Ventricular cardiac myocytes from 2-day-old neonatal Sprague-Dawley rats (Harlan, India-

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Address for reprint requests and other correspondence: L. Tung, Dept. of Biomedical Engineering, The Johns Hopkins Univ., 720 Rutland Ave., Baltimore, MD 21205 (E-mail: ltung@bme.jhu.edu).
nopolis, IN) were enzymatically dissociated using trypsin (US Biochemicals, Cleveland, OH) and collagenase (Worthington; Lakewood, NJ) and suspended in M199 culture medium (Life Technologies; Rockville, MD) supplemented with 10% fetal bovine serum (Life Technologies) as previously described (7). Approximately 7.5 × 10^5 cardiac myocytes were plated and grown as confluent monolayers on 20-mm-diameter fibronectin-coated glass or polyvinyl chloride (PVC) coverslips. Cell monolayers obtained in this fashion were isotropic, with no preferred direction in cell orientation. In actuality, cardiac tissue is structurally and biophysically anisotropic, with tissue properties such as conduction velocity and safety factor of conduction that depend on the direction of measurement. Therefore, experiments were also performed on anisotropic cell cultures using methods previously described (7). In brief, PVC coverslips were microabraded along a single direction with lapping paper before fibronectin coating and cell seeding. This resulted in cell monolayers having cells that were elongated and coaligned along the direction of abrasion. Experiments were conducted 5–8 days after cell plating.

Electrophysiological measurements. Individual cell monolayers were placed inside a Plexiglas chamber and superfused with warmed (35–37°C) oxygenated Tyrode solution (in mM: 135 NaCl, 5.4 KCl, 1.8 CaCl2, 1 MgCl2, 0.33 NaH2PO4, 5 HEPES, 5 glucose) for 5–10 min. The monolayer was then stained in the dark with 8 μM of the voltage-sensitive fluorescent dye, RH237 (Molecular Probes, Eugene, OR) for 5 min. Fluorescence signals were recorded using a contact fluorescence imaging method described previously (27). Action potentials were recorded from 61 sites arranged in a 17-mm-diameter hexagonal array. A 250-W quartz tungsten halogen lamp with an interference filter (530 ± 25 nm) delivered excitation light to the chamber via a liquid-filled light guide. The bottom of the chamber and the front end of the fiber bundle were painted with red ink (Avery Dennison; Brea, CA) that acted as an emission filter (long pass cutoff of 590 nm). Because dye staining can be nonuniform, optical signals from each recording site were each normalized to vary from their resting baseline (0%) to full action potential amplitude (100%). Photobleaching was not significant; a series of 2-s signals could be recorded up to eight times from a single location without appreciable loss of signal.

Mechanical stimulation apparatus. Mechanical stimuli were delivered by pulsing a vertical jet of Tyrode solution from a 0.4-mm-diameter nozzle located 1 mm above the cell monolayer (Fig. 1A). The Tyrode solution in the jet was prewarmed to 35–37°C and gated by a solenoid valve (The Lee Co., Westbrook, CT) controlled by a pulse generator (Fig. 1B). The jet velocity was varied by changing the air pressure inside the fluid reservoir, monitored with a differential pressure transducer (Honeywell, Morristown, NJ). The jet velocity at the nozzle tip (v) was calibrated by measuring the height (h) attained by the fluid jet when the nozzle was pointed up while the solenoid valve was opened for 10 ms (Fig. 1B):

\[

pgh = \frac{1}{2}mv^2 \quad (1)

\]

\[

v = \sqrt{2gh} \quad (2)

\]

where \( p \) is the fluid mass density and \( g \) is the gravitational constant.

For all experiments, the duration of solenoid valve opening was 10 ms. Other variables such as tubing size and the height of the fluid container relative to the nozzle were kept constant.

Characterization of mechanically induced extrasystoles. Cell monolayers with spontaneous activity exceeding 2 Hz (<10% of total number of cell monolayers) were not used for experiments. Monolayers were paced with monophasic rectangular pulses at 2 Hz (1.2× threshold, 10 ms) using a bipolar line electrode. Pulsed fluid jets were delivered to randomly selected sites while electrical pacing was turned off. A mechanical stimulus was considered to be successful if it generated an extrasystole and propagated activity. Attempts at mechanical stimulation were halted if they failed for three consecutive times. Jet velocities ranging from 0.3 to 1.8 m/s were tested, and velocities of 0.8–0.9 m/s were generally adequate to initiate propagated activity. In some experiments, mechanical stimulation was attempted for six times with different combinations of velocities and time waited between pulses (Δt). The average stimulation success rate (ASSR) was calculated as...
RESULTS

Mechanical point stimulation can trigger extrasystoles. In isotropic cultures, electrical (1.2× threshold, 10 ms) and mechanical (0.5–1.0 m/s, 10 ms) pulses initiated circular wavefronts that spread radially from the site of stimulus (Fig. 2). The conduction velocity of the mechanically induced wavefront deviated from the electrically induced wavefront by only 1.2 ± 2.5% (n = 10 monolayers). In anisotropic cultures, both electrical and mechanical point stimuli delivered to the same monolayer initiated an elliptical wavefront with a faster conduction velocity along the longitudinal direction (data not shown). Anisotropy ratio (AR), measured as the ratio of longitudinal to transverse conduction velocity, ranged from 1.7 to 2.7.

Mechanical excitation occurs without cellular injury. After successful mechanical stimulations, monolayers were tested for evidence of tissue damage in terms of regional slowing in conduction. Before delivering the mechanical stimulus, 2-Hz electrical pacing was applied with a bipolar line electrode at the edge of the monolayer. This resulted in a planar wavefront moving at constant velocity from left to right (Fig. 3, left). Mechanical point stimulation by itself initiated a wavefront that spread radially with constant velocity from the stimulus site (Fig. 3, middle). Triggered activity was never observed. Subsequent electrical stimuli again resulted in a planar wavefront moving at constant velocity, with no localized slowing in the mechanically stimulated area (Fig. 3, right).
To determine further whether the area subjected to the mechanical jet was damaged, we washed the monolayers with propidium iodide, a membrane-impermeable fluorescent stain for nucleic acid that is commonly used to visualize nonviable cells. Even for sites that were excited for more than six times, phase-contrast images did not show any structural damage and propidium iodide fluorescence images generally did not reveal an elevated number of nonviable cells in the mechanically stimulated areas. However, of 26 sites (in 12 monolayers) that were mechanically excited and studied afterwards, four sites (in 3 monolayers) did have a higher number of nonviable cells in the mechanically stimulated areas compared with neighboring regions. Jet velocities greater than 1.6 m/s induced cellular damage and holes in the monolayer that were evident in the phase-contrast images, but such velocities were clearly unnecessary for achieving mechanical excitation.

Mechanical excitations are highly variable. The total number of times a site could be mechanically excited was counted when the jet velocity was kept at 0.8–0.9 m/s and the time between successive stimulations (Δt) was kept at 1–2 min (Fig. 4). Of 62 sites in 25 monolayers, 10 sites (16.1%) could not be excited mechanically, although the remaining 52 sites (83.9%) were excited at least once. Among these excitable sites, 13 sites (21.0%) could be excited only once. A significant fraction of sites had a relatively high number of successful excitations. Nineteen sites (30.6%) were successfully stimulated for more than six times, and 11 of these sites (17.7% overall) were excited 10 times or more.

ASSR increases with jet velocity and with time between stimulations. Mechanical stimuli were delivered at jet velocities of 0.5, 0.8, and 1.0 m/s while Δt was kept constant at 1 min (Fig. 5). Generally, higher jet velocities induced extrasystoles with a higher probability of success. Increasing the velocity from 0.5 to 0.8 m/s increased ASSR by 18.6% and from 0.8 to 1.0 m/s increased ASSR by an additional 16.8%. The difference in ASSRs between the jet velocities of 0.5 and 1.0 m/s was statistically significant (P < 0.05). The importance of Δt was determined by delivering mechanical stimuli at 1-s, 10-s, 1-min, and 2-min intervals for six times while the jet velocity was fixed at 0.9 m/s (Fig. 5). Increasing Δt from 1 s to 10 s increased ASSR by an average of 12.8%, while increasing Δt from 10 s to 1 min increased ASSR by an additional 9.7% and from 1 min to 2 min increased ASSR by another 7.7%. These data could be fit with an exponential recovery curve having a time constant of 28 s. The differences in ASSRs between the Δtrs of 1 s and 1 min and between 1 s and 2 min were statistically significant (P < 0.05).

Our results also show that ASSR was 0.29 ± 0.07 (n = 25 sites, 16 monolayers) when Δt = 1 s and 0.60 ± 0.09 when Δt = 2 min. However, some sites were successfully excited with ASSR values close to 1, even with Δt = 1 s. In those sites, the monolayers could be mechanically excited repetitively at 1 Hz to generate propagated activities similar to those induced by 1 Hz electrical pacing with a point electrode.

SAC blockers suppress mechanical excitation. Gadolinium was administered to monolayers at concentrations between 20 and 100 μM for at least 10 min. Gd³⁺ (20 μM) prevented mechanical excitations without affecting the threshold of electrical excitation in six of nine (67%) sites. In the remaining three sites, the threshold for electrical excitation increased, whether or not mechanical excitation was prevented. Forty or 100 μM Gd³⁺ prevented mechanical excitations and also increased the threshold of electrical excitation (Table 1).

Additional tests of SAC activity were conducted with streptomycin. Mechanical stimuli with a jet velocity of 0.9 m/s were delivered six times to individual sites at 1-min intervals, and sites with ASSR >0.5 (average ASSR close to 0.9) were
selected so that changes in the mechanical responses could be observed more reliably. Six-minute exposure to 50 μM streptomycin decreased ASSR by 46% (Fig. 6) with statistical significance \((n = 16\) sites, 7 monolayers, \(P < 0.002\)). When the protocol was repeated in sham experiments using normal Tyrode solution, no significant change in ASSR was observed \((n = 9\) sites, 4 monolayers, \(P = 0.315\)). Administration of 10 μM nifedipine for at least 10 min also did not significantly change ASSR \((n = 6\) sites, 5 monolayers, \(P = 0.907\)), indicating that the streptomycin effect on ASSR was not mediated through block of L-type Ca\(^{2+}\) channels.

**Reentry can be induced by mechanical point stimulation.** Using the ability of fluid jets to induce extrasystoles, reentry induction was attempted (Table 2) using a paired S1-S2 pulse protocol \((49)\) used previously with only electrical stimuli. Of 230 attempts in 17 isotropic monolayers and 145 attempts in 9 anisotropic monolayers, one successful episode of reentry was obtained (Fig. 7). S1 stimulation from a point electrode initiated an elliptical wavefront (frame 2.031 s). After an S1-S2 coupling interval of 200 ms, the S2 mechanical point stimulus also generated an elliptical wave, but a unidirectional conduction block (frame 2.271 s, parallel lines) forced the wavefront to break and propagate around the blocked area (frames 2.311 s to 2.471 s). The waves then joined (frame 2.611 s) and continued to rotate in a figure-of-8 pattern. After another full rotation, the waves again merged (frame 3.011 s) but failed to complete another full rotation. Another episode of reentry was induced in an anisotropic monolayer with a mechanical point stimulus delivered to a spontaneously propagating wave (Fig. 8). The spontaneous activity generated a planar wavefront propagating from right to left (frame 0.230 s). Shortly afterward, a mechanical point stimulus was delivered (frame 0.310 s). This triggered an extrasystole (frame 0.350 s) and a region of conduction block (frame 0.350 s, parallel lines), resulting in a sustained single loop reentry (frame 0.430 s and later) that lasted for more than 18 rotations.

**DISCUSSION**

Many studies of mechanoelectric feedback in cardiac tissue have involved stretch of the ventricular free wall or the whole ventricle (20). These large-scale tissue experiments have demonstrated that mechanical pulsation can electrically stimulate cardiac tissue (18, 19). In this study, we showed that at a much smaller length scale, mechanical stimuli applied to two-dimensional monolayers of cardiac cells result in the activation and propagation of electrical wavefronts. Pulsatile fluid jets were directed perpendicular to the surface of the monolayer to produce localized “impacts” of force. Because the monolayer was bound to a rigid substrate, it allowed for relatively motion-free electrophysiological measurements by optical mapping. Consequently, our experiments were conducted in the absence of excitation-contraction uncouplers such as butanedione monoxime or cytochalasin D, which can alter tissue electrophysiology (41). Our results show that the fluid jet pulse can trigger action potentials that propagate electrically across the monolayer, just as in the case of electrical point stimulation. The likelihood for success of mechanical stimulation depends on 1) amplitude (jet velocity) of the stimulus, 2) time waited between

### Table 1. Effect of gadolinium (Gd\(^{3+}\)) on mechanical and electrical stimulations

<table>
<thead>
<tr>
<th>Mechanical Stimulation</th>
<th>Electrical Stimulation</th>
<th>20 μM</th>
<th>40 μM</th>
<th>100 μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>No effect</td>
<td>No change in threshold</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Became unexcitable</td>
<td>No change in threshold</td>
<td>6 (67%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>No effect</td>
<td>Increase in threshold</td>
<td>1 (11%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Became unexcitable</td>
<td>Increase in threshold</td>
<td>2 (22%)</td>
<td>2 (100%)</td>
<td>3 (100%)</td>
</tr>
</tbody>
</table>

The results from administration of 20, 40, and 100 μM Gd\(^{3+}\) were classified into 4 different possible categories depending on the way electrical and mechanical responses were affected.
stimuli, and 3) absence or presence of agents known to block stretch-activated channels (SACs). We also found that it is possible for mechanical stimulation to induce reentrant activity in these cell monolayers.

Impinging jet as a mechanical stimulus. Potent mechanical stimuli other than cell stretch operate in the heart. The laminar sheet structure of the myocardium results not only in sheet extension but also in sheet shear during systole (16). Intramyocardial pressure is distributed in a decreasing manner from endocardium to epicardium (2). Peak ventricular pressure rises immediately after impact associated with commotio cordis (37). These forms of mechanical stimuli may be conveniently applied to a tissue surface via an impinging fluid jet.

In a previous study on canine endothelium, histologically well-defined lesions were the result of a vertical fluid jet flowing out a 0.4-mm-diameter nozzle located 0.8 mm above the tissue surface, with jet velocity of 3.3–4.7 m/s and duration of 30 s (47). In our study, extrasystoles were induced without cellular injury by fluid jets of only 0.8–0.9 m/s and duration of 10 ms flowing out a 0.4-mm-diameter nozzle positioned 1 mm above the monolayer (Fig. 3). Fluid mechanical studies of submerged turbulent jets show that a fluid jet impinging on a planar surface consists of both pressure and shearing components (14). In the steady state, the pressure at the surface is greatest at the impingement point directly below the nozzle and decays rapidly with distance from the nozzle axis. In contrast, the shear stress is zero directly below the nozzle and peaks at a small radial distance away from the nozzle axis. Identifying the precise location of the origin of the mechanically induced wavefronts could help to determine if mechanically induced extrasystoles are caused by the compressive or by the shearing component of the impinging jet. However, the spatial resolution of our mapping system was inadequate to resolve the precise location of impulse formation and additional higher resolution experiments are required.

Because of the tethered nature of our cell cultures, the pressure and shear forces associated with the fluid jet do not stretch cells per se but involve cell deformations at a subcellular level, which are also capable of triggering action potentials and action currents (13, 25). Subcellular shear or compression can activate mechanosensitive currents in adult rat ventricular cells (5) and chick embryonic heart cells (6) and also decrease the L-type Ca current and inwardly rectifying potassium current in adult guinea pig ventricular cells (28). On the other hand, mechanical stresses in conjunction with regions of cell damage can initiate afterdepolarizations and action potentials via changes in intracellular Ca2+ (44). Our observations that extrasystoles could be induced without injury (Fig. 3 and results of propidium iodide experiments), that multiple extrasystoles were never observed, and that mechanically induced extrasystoles were suppressed by the SAC blockers Gd3+ (Table 1) and streptomycin (Fig. 6) support the notion that SACs may be involved in the mechanical response.

Variability of mechanical excitations. With the jet velocity at 0.8–0.9 m/s and time between mechanical stimulations (Δt) at 1–2 min, extrasystoles were induced at least once in 83.9% of the 62 sites tested (Fig. 4). The largest subgroup (21%) corresponding to sites that were excited only once, although a large percentage of sites (17.7%) could be excited 10 times or more. These observations suggest that different sites in the monolayers had varying sensitivities to the mechanical stimulus. Two distinct populations with different mechanical responses have been found in guinea pig ventricular myocytes (9) and it has been suggested that there may exist a small population of mechanically hypersensitive cardiac myocytes (42). Other experimental studies have shown that cardiac cells may acquire mechanosensitivity via coupling to mechanically sensitive fibroblasts (29). Thus regional variations in cardiac cell mechanosensitivity may account for the variability in ASSR in our cell cultures.

Effect of jet velocity and time between mechanical stimulations. With Δt kept constant at 1 min, a graded increase in jet velocity from 0.5 to 1.0 m/s produced a graded increase in ASSR (Fig. 5). This result is in agreement with observations in rabbit (19), dog (22), and frog hearts (18), where the probability of a stretch to induce premature ventricular excitations increased with increasing rate and increasing extent of stretch. In studies of projectile impact on pig hearts, the incidence of ventricular fibrillation increased with increasing projectile ve-
locities, although it reached a maximum and decreased with further increases in velocity (38).

ASSR was also affected by the time waited between mechanical stimulations ($\Delta t$). After a mechanical stimulus, the mechanical excitability immediately dropped below its pre-stimulus level, but recovered with a time constant of 28 s. Mechanical adaptation similar to our results was observed during the induction of premature ventricular beats by a sudden inflation of rabbit ventricles (15). In those experiments, the induction of premature ventricular beats was reduced at small $\Delta t$ but completely recovered with $\Delta t$ of 1 min. The “mechanoelectric adaptation period” was attributed to accumulation of $K^+$ in the restricted extracellular space and to intracellular accumulation of $Ca^{2+}$. Alternatively, adaptation to mechanical stimuli over second-long time scales has been shown in whole cell currents of single chick embryonic cells (6) and in single channel recordings of adult rat atrial cells (39). Interestingly, mechanosensitivity reappeared in the chick cells with the application of fluid flow across the cells and was attributed to the washout of autocrine inhibitory factors released by the cells (6). Thus the exact mechanism of mechanical adaptation in cardiac tissue still remains largely speculative.

Fig. 7. Mechanical induction of figure-of-8 reentry. In an anisotropic monolayer, an electrical point stimulus at the * (frame 2.031 s) initiated an elliptical wavefront. At frame 2.191 s, a mechanical point stimulus was applied at the circled *. This triggered an extrasystole with unidirectional block (frame 2.271 s), which evolved into nonsustained figure-of-8 reentry. Arrows indicate directions of wave front propagation.

Fig. 8. Mechanical induction of sustained single loop reentry. In an anisotropic monolayer, spontaneous activity initiated a planar wave propagating from right to left. At 0.310 s, a mechanical point stimulus was applied at the circled *. This triggered an extrasystole with unidirectional block (frame 0.390 s), which evolved into sustained single loop reentry. Arrows indicate directions of wave front propagation.
Effect of SAC blockers. Gadolinium has been widely used as an agent to block SACs in cardiac cells (8, 23, 26). Supplementation with 20 μM Gd3+ prevented mechanical excitation without affecting the threshold of electrical excitation in six of nine sites tested (Table 1). Higher concentrations of Gd3+ prevented mechanical excitation but also increased the threshold of electrical excitation, perhaps through inhibition of I_{Na} (36). Gadolinium can also block other channel types, including the L-type Ca channel (35), Ca-activated K-ATP channel (30), and store-operated channels (46). Therefore streptomycin, another SAC blocker thought to act on cardiac cells (21, 28), was also tested. ASSR decreased by 46% with 50 μM streptomycin, without a change in electrical excitation threshold. Because Gd3+ and streptomycin are also known to block L-type Ca2+ currents (4, 35), additional experiments were conducted with 10 μM of the Ca channel blocker nifedipine. No significant change in ASSR was observed (Fig. 6). Thus these results strongly suggest that the mechanically induced extrasystoles observed in our experiments involve SACs.

Mechanical induction of reentry. In the literature of reentrant waves that are electrically induced, Winfree (49) described a hypothetical “pinwheel” experiment, whereby reentry is initiated by an appropriately timed electrical point stimulus (S2) applied during the refractory tail of a paced (S1) wavefront. This electrical protocol can lead to reentrant activity in intact cardiac tissue (10) and cardiac cell cultures (27). In this study, a fluid jet pulse acted as the S2 stimulus. However, only one episode of reentry was successfully obtained using a programmed S1-S2 protocol (Fig. 7), out of 145 attempts in 9 anisotropic monolayers and 230 attempts in 17 isotropic monolayers (Table 2). A second episode of reentry occurred as the result of mechanical stimulation applied in the wake of a spontaneously propagating wave (Fig. 8). We believe that reentry was difficult to induce for several reasons. First, the vulnerable window of reentry induction may be very narrow, and for electrical stimuli it is only a few milliseconds long (27). Second, mechanical stimuli cannot be applied as quickly as can electrical pulses. Because of inertial effects, it takes time for the fluid jet to become fully developed. A slowing of the rate of rise of a mechanical stimulus is known to diminish the mechanical induction of extrasystoles (18, 19). Finally, the total number of extrasystoles that can be mechanically induced at a given site is limited (Fig. 4) so that it is difficult to test many different S1-S2 coupling intervals.

Potential limitations of the study. One potential limitation of our study is whether the mechanical perturbations may be affecting small numbers of pacemaker cells that may have been present in the cell monolayers (although we isolated cells from only the bottom 70% of the ventricle). It may be argued that the pacemaking ability of these cells would be suppressed by the large electrical load presented by surrounding ventricular cells but that mechanical perturbations may augment the pacemaker activity, as underlies the Bainbridge reflex to stretch and has been observed in isolated sinoatrial nodal tissue (1) and isolated sinoatrial nodal cells (11). However, in the former study, the tissue was stretched by an unspecified length for a period of 5 s, whereas in the latter study cell length was increased by 5–10% of resting length and held for the duration of measurements (time interval unspecified). In our experiments, there was no change in cell length, and the fluid jet pulse lasted for only ~10 ms. Therefore, the mechanical responses in our experiments are unlikely to be via the same mechanisms.

A final cautionary note relates to the neonatal rat cardiomyocytes that make up our cell cultures. Our choice of these cells is constrained by the need for mammalian cardiac cells that can remodel to form confluent monolayers. However, the cells have an electrophysiology that differs significantly from ventricular cells from the human (shorter action potential duration, less prominent plateau, slower conduction velocity for neonatal rat) and from adult rat cells. Nonetheless, this model system has proven to be useful in the study of cardiac conduction, hyper trophy, ischemia reperfusion, and, in the context of MEF, the possible role of stretch-activated channels in the genesis of arrhythmia.

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GRANTS

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MECHANO-ELECTRICAL FEEDBACK IN CARDIAC CELL CULTURES