Fast pacing facilitates discontinuous action potential propagation between rabbit atrial cells

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Fast pacing facilitates discontinuous action potential propagation between rabbit atrial cells. Am J Physiol Heart Circ Physiol 279: H2095–H2103, 2000.—We examined the critical coupling conductance (\(G_C\)) for propagation at different pacing cycle lengths (CLs) (1,000 and 400 ms). As \(G_C\) was progressively reduced, propagation failed at a CL of 1,000 ms, whereas propagation succeeded at a CL of 400 ms over a range of \(G_C\) values before failing at a CL of 400 ms at a lower \(G_C\), showing facilitation of propagation at the shorter CL. Critical \(G_C\) was (means ± SE) 0.8 ± 0.1 nS for a CL of 400 ms and 1.3 ± 0.1 nS for a CL of 1,000 ms (a 63% increase, \(P < 0.002, n = 9\) cell pairs). In 14 uncoupled cells, action potential duration at 30% repolarization (APD30) increased from 19.9 ± 2.5 to 41.8 ± 2.6 ms (\(P < 0.001\)) as CL decreased from 1,000 to 400 ms. In five cell pairs, critical \(G_C\) with 4-amino-pyridine (4-AP) was reduced to 0.4 ± 0.1 nS at a CL of 1,000 ms (\(P < 0.05\) compared with control solution), and critical \(G_C\) in 4-AP was unchanged by decreasing CL to 400 ms. It is possible that the “remodeling” of atrial cells due to atrial fibrillation or tachycardia, which has been shown to produce a decrease in the transient outward current, may result in an enhanced ability to propagate, possibly facilitating further development of fibrillation under conditions of decreased cellular coupling.

ATRIAL ARRHYTHMIAS FREQUENTLY OCCUR either with or without a preexisting myopathy associated with atrial dilatation from ventricular disease or valvular dysfunction (4, 22). In the National Institutes of Health-sponsored Cardiovascular Health Study of 5,201 people over the age of 60 yr, 4.8% of women and 6.2% of men had atrial fibrillation (4). In studies of atrial arrhythmias (11, 12, 19, 26), there has been a considerable emphasis on the complex three-dimensional anatomic structure of the atrium in which many regions of slowed conduction, which may be discontinuous, have been described. There is also a well-known frequency dependence of the initiation of atrial fibrillation with atrial tachycardia, perhaps associated with atrioventricular reentry, “degenerating” into atrial fibrillation (20). Lesh et al. (17) discussed the importance of discontinuous conduction in many clinical atrial arrhythmias, identifying the crista terminalis (CT) as a major factor in both reentrant and focal arrhythmias, primarily due to its marked electrical anisotropy (21). Recent work (19) has shown that the CT and eustachian ridge may form the posterior barrier in human atrial flutter. In normal atria, the CT has poor transverse coupling, but in patients with flutter, there is nearly complete uncoupling (either congenital or acquired) along the length of the CT. Atypical flutter may only use a portion of the CT to form the line of block, and these lines of block may be partly functional and develop at faster rates. Focal atrial arrhythmias are also likely to arise from the CT because the poor coupling protects an ectopic focus from the surrounding electrotonic influences of the quiescent atria, which would tend to suppress the automaticity. Kalman et al. (15) found that 14 of 18 focal right atrial tachycardias arose from the CT, with fractionated electrograms indicating discontinuous conduction. Atrial fibrillation may result from several different mechanisms, including rapid focal activity that cannot propagate in a 1:1 fashion to the atria or rapid reentry around multiple uncoupled regions. The CT may be a region of uncoupling around which the reentrant wave may circulate. Spach et al. (25) demonstrated that premature stimuli produced unidirectional block and microreentry in isolated nonuniformly anisotropic human atrial trabeculae from older patients but smooth propagation in trabeculae from younger patients, and similar results in vivo have been reported (17). There may be a spectrum of the degree and extent of discontinuous conduction so that typical atrial flutter occurs with uncoupling along the entire CT and eustachian ridge, paroxysmal “flutter/fib” may occur with only a small portion of the CT having discontinuous conduction, and very fine atrial fibrillation may occur at the cellular level with cell-to-cell uncoupling distributed through many regions of the atrium, allowing multiple wavelets to exist (17).
A coupling clamp technique (see Refs. 27 and 28) allows the electrical coupling of real isolated heart cells with a controlled value of coupling conductance ($G_C$). When two cells are coupled with a relatively low $G_C$, the conduction delay between the two cells becomes prolonged and is described as discontinuous conduction. The action potential shape of atrial cells is fundamentally different from that of ventricular cells, particularly due to the presence of a rapid early repolarization in atrial cells, which is largely produced by the activation of a transient outward current ($I_o$) (5). We (14, 27) showed previously that the amplitude of the early plateau was important in ventricular cells in allowing discontinuous conduction because the membrane potential during this early plateau supplies the current for charging the distal cell(s). Because the $I_o$ of atrial cells has been shown to be frequency dependent, with less $I_o$ at higher frequencies of pacing, we hypothesized that at increased pacing frequencies, a pair of atrial cells would have successful propagation at values of $G_C$ that did not allow successful propagation at lower pacing frequencies.

**METHODS**

Cell isolation and electrodes. Single atrial myocytes were prepared from adult New Zealand White rabbits weighing 2.5–3.5 kg. The rabbits were anesthetized using 50 mg/kg iv pentobarbital sodium and 500 U iv heparin, the heart was rapidly extracted via thoracotomy with artificial respiration, and the aorta was cannulated for Langendorff perfusion. Single cells were isolated according to the methods described previously by Hancox et al. (8). Briefly, the cannulated heart was perfused sequentially at 37°C with base solution + 750 μM CaCl$_2$ for 5 min, base solution + 100 μM EGTA for 5 min, and base solution + 240 μM CaCl$_2$ + enzyme for 5 min (see Solutions). The interatrial septum was then excised, cut into thin strips, and further digested in the recirculated enzyme solution used above for 6 min. Cells were isolated by trituration the tissue strips and were then placed in a potassium glutamate solution for 1 h at room temperature.

The cells were placed in a chamber that was continuously perfused with Tyrode solution at 2 ml/min and that always maintained the temperature at 35 ± 0.5°C. The pipettes were pulled from borosilicate glass and, after fire polishing, had resistances of 3–4 MΩ when filled with the internal solution. High-resistance seals were formed with the cell membrane by applying light suction, and the membrane was disrupted by applying transient suction. The junctional potential was corrected by zeroing the potential before the pipette tip touched the cell membrane.

**Solutions.** The base solution contained (in mM) 130 NaCl, 4.5 KCl, 3.5 MgCl$_2$, 0.4 Na$_2$HPO$_4$, 5 HEPES, and 10 dextrose at pH 7.25. The enzyme solution contained 1 mg/ml collagenase (type IIA, Worthington), 0.07 mg/ml protease (type XIV, Sigma), and base solution + 240 μM CaCl$_2$. The potassium glutamate solution had (in mM) 100 potassium glutamate, 25 KCl, 10 KH$_2$PO$_4$, 0.5 EGTA, 1 MgSO$_4$, 20 taurine, 5 HEPES, and 10 dextrose at pH 7.2. The normal Tyrode solution contained (in mM) 148.8 NaCl, 4 KCl, 1.8 CaCl$_2$, 0.53 MgCl$_2$, 0.33 Na$_2$HPO$_4$, 5 HEPES, and 5 dextrose at pH 7.4. The internal solution was composed of (in mM) 135 KCl, 5 disodium creatine phosphate, 5 MgATP, and 10 HEPES at pH 7.2.

**Electrical coupling of atrial cell pairs.** Suguira and Joyner (27) developed an electrical circuit that can provide a variable $G_C$ between two isolated myocytes that are not actually in contact with each other. We define $V_1$ as a time-varying membrane potential of cell 1 and $V_2$ as a time-varying membrane potential of cell 2. If the two cells were coupled together by an intercellular conductance $G_C$, there would be a time-varying current ($I_v$) flowing from cell 1 to cell 2 given by $I_C = (V_1 - V_2) \times G_C$. We used a 500-MHz Pentium III PC computer (Gateway) with a fast analog-to-digital and digital-to-analog (Digidata 1200, Axon Instruments, Foster City, CA) system to compute the value of $I_C$ from the sampled values of $V_1$ and $V_2$ and a selected value of $G_C$ at time intervals of less than 80 μs. This value of $I_C$ is then added to cell 2 and subtracted from cell 1 (added with a negative sign) to produce the effect of the desired value of $G_C$. Stimulation current pulses of a 2-msec duration are also added to cell 1 and/or cell 2 at defined cycle lengths. Membrane potentials were recorded with an Axoclamp 2A dual amplifier (Axon Instruments), in the current clamp mode as previously described (27), using the internal voltage-to-current converters to feed back the desired currents to each headstage. Series resistance was carefully compensated by internal bridge balance adjustments after recording of the membrane potential was established.

**Statistical analysis.** Statistical analysis was performed with SigmaStat for Windows (Jandel Scientific). Statistical significance was determined by Student's $t$-test. $P$ values <0.05 were regarded as significant. Data are presented as means ± SE in the text.

**RESULTS**

To examine the propagation of action potentials between two atrial cells at two different cycle lengths (CLs) for stimulation, we followed a protocol in which we established a certain value of $G_C$ between two cells, paced one of the cells of the cell pair repetitively for 15 stimulations at a CL of 1,000 ms, followed by 25 stimulations at a CL of 400 ms, and then another 10 stimulations at a CL of 1,000 ms. We subsequently changed the value of $G_C$ and repeated the stimulation protocol. At high levels of $G_C$, we found that propagation was successful for all cell pairs at both CLs. This phenomenon is illustrated in Fig. 1, in which we show membrane potential recordings from the stimulated cell (top) and the follower cell (middle) and the coupling current (lower) with $G_C = 1.0$ nS. The recordings show the last three stimulations at a CL of 1,000 ms, 25 stimulations at a CL of 400 ms, and the first four stimulations after the return to a CL of 1,000 ms. All of the action potentials are propagated successfully, but a closer examination of the action potential properties shows some significant differences at the two values of CL. Figure 2 (top) shows, at a faster time scale, the action potentials of the leader cell ($V_1$) and the follower cell ($V_2$) for the action potentials at the last stimulation at a CL of 1,000 ms and the 13th stimulation at a CL of 400 ms of Fig. 1. For the leader cell, the action potential amplitude is nearly the same for the two values of CL, but the early repolarization occurs much more quickly at a CL of 1,000 ms than at a CL of 400 ms. The slowed early repolarization at a CL of 400 ms produces a greater voltage difference between the leader cell and
the follower cell during the propagation process. As shown in Fig. 2 (bottom), which plots the coupling current (positive in the direction from the leader cell to the follower cell), the peak value of coupling current is the same at the two values of CL, but the decline in coupling current is much slower for a CL of 400 ms. Thus the charge transferred from the leader cell to the follower cell (the time integral of the coupling current), which produces the depolarization of the follower cell, occurs more quickly at a CL of 400 ms than at a CL of 1,000 ms. Therefore, the conduction delay between the leader cell and the follower cell is decreased.

This effect occurs as soon as the CL is changed from 1,000 to 400 ms and rapidly reverses when the CL is changed back to 1,000 ms, as shown in Fig. 3. Figure 3 (top) shows the action potential duration at 30% repos...
larization (APD$_{30}$) values for the leader cell for the last three action potentials at a CL of 1,000 ms, 25 stimulations at a CL of 400 ms, and the first three action potentials after the switch back to a CL of 1,000 ms. Note that the APD$_{30}$ is increased for all of the stimulations at the shorter CL. Figure 3 (bottom) shows the conduction delay (the elapsed time from the upstroke of the leader cell to the upstroke of the follower cell) for the same action potentials, with a decreased conduction delay for all of the stimulations at a CL of 400 ms compared with a CL of 1,000 ms. The shorter conduction delay at a CL of 400 ms compared with a CL of 1,000 ms suggests that conduction is facilitated at the shorter value of CL. As we decreased $G_C$ from 1.0 to 0.9 nS and repeated the same protocol on the same cell pair, we got the results shown in Fig. 4. The last three stimulations at a CL of 1,000

**Fig. 4.** Recordings of membrane potential of the leader cell (top) and the follower cell (middle) of the same atrial cell pair as for Fig. 1 but now coupled with $G_C = 0.9$ nS as the CL for stimulation is changed from 1,000 to 400 ms and then back to 1,000 ms. The data shown are the last three stimulations at a CL of 1,000 ms, 25 stimulations at a CL of 400 ms, and the first four stimulations after the return to a CL of 1,000 ms. Bottom: coupling current plotted as positive in the direction from the leader cell to the follower cell. *Successful propagation of the first 2 action potentials after the switch back to a CL of 1,000 ms.
ms all show conduction failure, as indicated by the full amplitude action potential in the leader cell (top) and the much smaller passive response in the follower cell (middle). However, all of the stimulations at a CL of 400 ms show successful propagation from the leader cell to the follower cell. The effects of the changes in CL are not instantaneously manifested. Note that the first two action potentials after the return to a CL of 1,000 ms show propagation, although the third and fourth action potentials after this transition fail to propagate, as did the subsequent action potentials at this CL (data not shown). There is also a gradual transition in the propagation characteristics when CL is changed from 1,000 to 400 ms, as illustrated in Fig. 5. In Fig. 5, the membrane potential of the leader cell and the follower cell are plotted along with the corresponding coupling current. The first stimulation shown is the last of the stimulations at a CL of 1,000 ms, followed by the first three stimulations at a CL of 400 ms. Note that the first stimulation shown results in propagation failure, and thus the coupling current is entirely positive (flowing from the leader cell to the follower cell). For the subsequent three stimulations, there is propagation success, and the coupling current has a reversal of direction resulting in both a positive and negative phase. Interestingly, the APD30 of the action potential of the follower cell progressively increases for each successful activation. Because the follower cell was not activated during the period of a CL of 1,000 ms, it has a large Ito (and thus a very rapid early repolarization) for the first successful propagation. Ito decreases over several successful activations, resulting in a gradual increase in APD30 until a steady state is reached. Figure 6 shows the transition from a CL of 400 ms to a CL of 1,000 ms at GC = 0.9 nS. For this figure, the first stimulation shown is the last stimulation at a CL of 400 ms, followed by the first three stimulations after the return to a CL of 1,000 ms. The three stimulations at a CL of 1,000 ms show a progressive prolongation of conduction time to conduction failure for the third stimulation at a CL of 1,000 ms as the APD30 of the follower cell progressively decreases.

As we further reduced GC for this cell pair, repeating the protocol, we found that propagation was successful at a CL of 400 ms at a GC value as low as 0.65 nS but failed at GC = 0.6 nS, as illustrated in Fig. 7. Figure 7 (top) shows the membrane potential for the leader cell and the follower cell during steady-state propagation at a CL of 400 ms, with each stimulation producing successful propagation. Figure 7 (bottom) shows a recording from the same time period at a CL of 400 ms stimulation for GC = 0.6 nS, showing that each stimulation now results in failure of propagation. In nine cell pairs, the mean value of critical GC for successful propagation decreased from 1.3 ± 0.1 nS at a CL of 1,000 ms to 0.8 ± 0.1 nS at a CL of 400 ms (P < 0.002). Compared with the critical GC at a CL of 400 ms, the increase in CL to 1,000 ms required a 63% increase in the critical value of GC required for propagation, demonstrating that conduction was facilitated at a CL of 400 ms compared with that at a CL of 1,000 ms.

The increase in APD30 by shortening the CL is not produced by the propagation process. This is illustrated in Fig. 8 (top), which shows the membrane potential for a single isolated atrial cell in which the stimulus CL is changed from 1,000 to 400 ms. The data plotted show the last stimulation at a CL of 1,000 ms and the first three stimulations at a CL of 400 ms. The

![Fig. 5. Replotting of the data of Fig. 4 at a faster time base for the transition from a CL of 1,000 ms to a CL of 400 ms. Top: superimposed membrane potentials of the leader cell (solid line) and the follower cell (dotted line). Bottom: coupling current for the last stimulation at a CL of 1,000 ms followed by the first three stimulations at a CL of 400 ms.](http://ajpheart.physiology.org/Downloadedfrom)
APD$_{30}$ of the action potentials is closely approximated by the time that each action potential is above the 0 mV line and this is clearly increased at the short CL value. When we exposed this cell to 2 mM 4-AP to block $I_h$ and repeated the stimulation protocol, we obtained the data shown in Fig. 8 (bottom). The APD$_{30}$ is clearly increased at a CL of 1,000 ms [compared to the control solution, Fig. 8 (top)] and is now not further increased by switching the CL to 400 ms. When we analyzed APD$_{30}$ for isolated atrial cells (no coupling) at a CL of 1,000 and 400 ms, we found an increase in APD$_{30}$ in the control solution from 19.9 ± 2.5 to 41.8 ± 2.5 ms (an increase of 110%, n = 14, P < 0.001). In the 2 mM 4-AP solution, the mean APD$_{30}$ values were 60.9 ± 5.2 ms (CL of 1,000 ms, n = 12, P < 0.001 compared with a CL of 1,000 ms in control solution) and 62.8 ± 5.7 ms (n = 12, CL of 400 ms, not significantly different from the APD$_{30}$ values at a CL of 1,000 ms in 4-AP).

These results with 4-AP suggested that propagation for cell pairs in the 4-AP solution would have a lower...
critical $G_C$ and show less frequency dependence of propagation success than those in the control solution. This is illustrated in Fig. 9, which shows data from a coupled atrial cell pair exposed to 2 mM 4-AP and stimulated at a CL of 1,000 ms. Figure 9 (top) shows a transition from a CL of 1,000 ms to a CL of 400 ms with $G_C = 0.4$ nS, with all of the stimulations of the leader cell followed by activation of the follower cell at both values of CL. For the same cell pair, with $G_C = 0.3$ nS [Fig. 9 (bottom)], failure of propagation occurs at a CL of 1,000 ms and at a CL of 400 ms. Thus the presence of 4-AP lowers the critical $G_C$ for action potential conduction (in this case to between 0.3 and 0.4 nS) compared with the control solution and removes the frequency dependence of conduction. For five cell pairs exposed to 2 mM 4-AP, the critical value of $G_C$ required...
to sustain conduction at a CL of 1,000 ms was 0.4 ± 0.1 nS ($P < 0.05$ compared with control solution, CL of 1,000 ms), and the critical $G_c$ in the 4-AP solution was unchanged by decreasing CL to 400 ms.

**DISCUSSION**

Sugiura and Joyner (27) previously showed that for discontinuous conduction in guinea pig ventricular cell pairs, decreases of the stimulation CL from 500 ms to 400, 300, 200, 190, 180, and 160 ms could maintain consistent 1:1 discontinuous conduction from cell 1 to cell 2, but the conduction delay progressively increased as the CL was decreased and block occurred at CL 130 ms as 2:1 conduction. Sugiura and Joyner (27) also showed that 1 µM nifedipine significantly increased and isoproterenol significantly decreased the delay between the activation of two coupled cells at a given $G_c$, suggesting that the primary determination of the increased conduction delay at a shorter CL was the residual inactivation of the L-type calcium current. In contrast to these results with guinea pig ventricular cells, the present results with rabbit atrial cells show a very different phenomenon with a change in CL from 1,000 to 400 ms actually producing a shorter conduction delay and a greater safety factor (requiring less $G_c$) for conduction. One major difference in these two cell types is the dominant effects of $I_w$ in atrial cells on the early repolarization after the spike of the action potential. When the cycle length decreases, $I_w$ during the early repolarization phase will decrease due to the incomplete recovery from inactivation. This shifts the balance of membrane current during this phase toward less outward current and thus slows this early repolarization phase, increasing APD$_{90}$ and allowing more current to flow from the leader cell to the follower cell, which allows the follower cell to more quickly reach its voltage threshold for action potential initiation. This action decreases the conduction delay and facilitates the propagation at short cycle lengths.

Numerous studies have demonstrated the variable magnitude of $I_w$ (5) in different species and in different regions of the heart. $I_w$ has been recorded in a wide range of cardiac tissues, including ventricular cells from the rat (31), ferret (2), dog (18), and rabbit (9), atrial cells from the rabbit (3) and dog (33), and atrial and ventricular cells from humans (24, 34) and rabbits (7), with variable densities and kinetics. $I_w$ can also be altered in physiological and pathophysiological conditions. For example, it has been shown that $I_w$ has differences in both density and kinetics in human sub-endocardial versus subepicardial ventricular cells (34). There are also age-related changes (32), and alterations in pathological conditions such as myocardial infarction or ischemia (13), cardiac hypertrophy (29), terminal heart failure, and atrial dilatation (1, 16). Interactions between coupled myocytes during the early plateau phase were studied by Huelsing et al. (10), showing that the greater $I_w$ magnitude of rabbit Purkinje cells compared with rabbit ventricular cells produced an intrinsically more rapid repolarization of the Purkinje cell and thus caused complex interactions during the early repolarization period, but in these experiments the cells were simultaneously stimulated such that propagation of the action potential was not studied. Although many of these studies have focused on either the magnitude of the current or the duration of the action potentials, few previous studies have focused on the effects of $I_w$ on action potential propagation. Because action potential propagation between two adjacent cardiac cells with high levels of $G_c$ occurs with very short delays, the activation of $I_w$ does not play a role in propagation under these conditions, because conduction occurs before activation of $I_w$. However, the long delays associated with discontinuous conduction (with lower levels of $G_c$) make early plateau currents, such as the L-type calcium current and $I_w$, important components of the propagation process.

Our findings may have implications for clinical arrhythmias under pathophysiological conditions. In regions of the atrium with low $G_c$, some premature beats may be conducted with decreased delay and require less $G_c$. This could facilitate the initiation of atrial arrhythmias because the premature beat may be able to propagate along a pathway that was blocked for the regular excitations, thus changing the spatial pattern of the activation wavefront. $I_w$ was found to be substantially decreased in atrial cells from patients with atrial fibrillation (30) and in ventricular cells from patients with heart failure (1). The decreased $I_w$ in the atrial cells after atrial tachycardia or fibrillation may actually facilitate action potential conduction at regions of low $G_c$ and may encourage the re-initiation of atrial tachycardia or fibrillation. In addition, the lower $I_w$ in human subendocardial compared with subepicardial ventricular cells (34) and the decreased $I_w$ after myocardial infarction or ischemia (13) may be important in the initiation of the ventricular action potential at Purkinje-ventricular junctions and may facilitate ventricular discontinuous conduction.

The limitations of the study are the following. Our experiments were performed with rabbit atrial cells. Although the ionic properties in rabbit atrial cells are similar to human atrial cells, the $I_w$ recovery time course was found to be faster in human atrial cells than in rabbit atrial cells (6). Further experiments need to be done to understand whether this frequency-dependent facilitation of propagation happens in human atrial cells. Action potential propagation within any electrical syncytium, even if well coupled, can be described at a microscopic level as discontinuous because propagation across the gap junctions takes more time than propagation across an equivalent length of cytoplasm within the cell. However, the type of discontinuous conduction we are studying with our coupling clamp technique is specifically that found at regions such as the Purkinje-ventricular junction or in post-ischemic tissue, in which there is a clearly measurable delay (on the order of 4–30 ms) between the activations of closely adjacent groups of cells with electrotonic prepotentials in the distal cells. At large values of $G_c$, in which the conduction delay between adjacent cells...
becomes on the order of 1 ms or less, any process (such as the L-type calcium current or $I_{Ca}$) that develops with a delay of several milliseconds after the upstroke of the action potential cannot directly alter propagation, which we showed experimentally with modulation of L-type calcium current by nifedipine (27) and which has been shown theoretically with strand simulations (23).

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