Electrotonic load triggers remodeling of repolarizing current $I_{to}$ in ventricle

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Libbus, Imad, Xiaoping Wan, and David S. Rosenbaum. Electrotonic load triggers remodeling of repolarizing current $I_{to}$ in ventricle. *Am J Physiol Heart Circ Physiol* 286: H1901–H1909, 2004. First published January 8, 2004; 10.1152/ajpheart.00581.2003.—A change in activation sequence electrically remodels ventricular myocardium, causing persistent changes in repolarizing currents (T-wave memory). However, the underlying mechanism for triggering activation sequence-dependent remodeling is unknown. Optical action potentials were mapped with high resolution from the epicardial surface of the arterially perfused canine wedge preparation ($n = 23$) during 30 min of baseline endocardial stimulation, followed by 40 min of epicardial stimulation, and, finally, restoration of endocardial stimulation. Immediately after the change from endocardial to epicardial stimulation, phase 1 notch amplitude of epicardial cells was attenuated by $74 \pm 8\%$ ($P < 0.001$) compared with baseline and continued to diminish during the period of epicardial pacing, suggesting progressive remodeling of the transient outward current ($I_{to}$). When endocardial pacing was restored, notch amplitude did not immediately recover but remained attenuated by $23 \pm 10\%$ ($P < 0.001$), also consistent with a remodeling effect. Peak $I_{to}$ current measured from isolated epicardial myocytes changed by $12 \pm 4\%$ ($P < 0.025$), providing direct evidence for $I_{to}$ remodeling occurring on a surprisingly short time scale. The mechanism for triggering remodeling of $I_{to}$ was a significant reduction (by $14 \pm 4\%$, $P < 0.001$) of upstroke amplitude in epicardial cells during epicardial stimulation. Reduction in upstroke amplitude during epicardial pacing was explained by electrotonic load on epicardial cells by fully repolarized downstream endocardial cells. These data suggest a novel mechanism for triggering electrical remodeling in the ventricle. Electrotonic load imposed by a change in activation sequence reduces upstroke amplitude, which, in turn, attenuates $I_{to}$ according to its known voltage-dependent properties, triggering downregulation of current.

cardiac memory; electrical remodeling; optical mapping

ELECTRICAL REMODELING is a persistent change in the electrophysiological properties of myocardium in response to a change in rate or activation sequence. It is well established that electrical remodeling alters the electrophysiological properties of atrial tissue and susceptibility to atrial fibrillation (AF) (31, 33, 34, 36, 37). Atrial remodeling is associated with changes in ion channels at the cellular level, such as a reduction in the transient outward potassium current ($I_{to}$) and L-type calcium current. However, the fundamental mechanism responsible for triggering changes in channel expression in response to alterations in rate and activation sequence in AF is poorly understood. Even less is known about the process of electrical remodeling in the ventricle. Ventricular pacing and periods of tachycardia cause T-wave changes ascribed to “cardiac memory” and “posttachycardia T-wave changes,” respectively, which are undoubtedly manifestations of remodeling of ventricular repolarization (6, 12, 24, 26, 27). After periods of atrial arrhythmias with rapid ventricular response rate, restoration of physiological ventricular rates by atrioventricular node ablation and pacemaker implantation is associated with bradycardia-dependent ventricular arrhythmias, suggesting that remodeling of ventricular myocardium can enhance susceptibility to arrhythmias (5, 8).

Several previous studies have begun to address the ionic basis of electrical remodeling in the ventricle (9, 14, 35). After 3- to 4-wk periods of ventricular pacing, Yu et al. (35) demonstrated a significant reduction in $I_{to}$, which was reflected in the attenuation of the action potential notch and prolongation of action potential duration (APD). In contrast, comparable periods of ventricular pacing did not alter sodium current and L-type calcium current (14), further supporting the hypothesis that remodeling of ventricular repolarization is attributed to reduced expression of outward potassium currents in general and of $I_{to}$ in particular.

Previous data suggest that it is the change in activation sequence rather than elevated heart rate which most substantially leads to electrical remodeling of the ventricle (9, 19). However, there remains a major unresolved question: why would the expression or activity of ionic currents, such as $I_{to}$ be influenced by the direction of depolarization? What are the cell signaling pathways or triggers that regulate the expression of ionic current in response to changes in the sequence of propagation? Passive electrotonic coupling between cells can impose an electrical load on myocytes during propagation, which can produce changes in transmembrane potential independent of active ionic processes (28). During the early plateau phase of the action potential, when there is a critical balance between depolarizing and repolarizing currents, even small changes in transmembrane potential can cause significant changes in activation of voltage-dependent currents such as $I_{to}$ (13). In the present study, epicardial and transmural optical mapping was used to demonstrate a novel mechanism for triggering electrical remodeling. Specifically, we hypothesized that a change in activation sequence imposes an electrotonic load on myocardium, resulting in a reduction of action potential upstroke amplitude and triggering downregulation (i.e., remodeling) of the voltage-dependent repolarizing current $I_{to}$.

MATERIALS AND METHODS

The animal care protocols in this study followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals.
**Canine wedge preparation.** To assess cell-to-cell interaction and action potential changes underlying remodeling, we developed a system for optically mapping action potentials across the epicardial surface and transmural wall of the arterially perfused canine wedge preparation (3a). Briefly, hearts were excised from 23 male mongrel dogs weighing 20–25 kg. Wedges of myocardium measuring \(3 \times 1.5 \times 1\) cm were dissected from the anterior, anterolateral, or posterior free walls of the canine left ventricle (LV) surrounding secondary branches of the circumflex and left anterior descending coronary arteries (\(n = 23\)) and from the right ventricle (RV) surrounding secondary branches of the right coronary artery (\(n = 9\)). Wedges were perfused through a plastic cannula inserted into the small (100 \(\mu\)m) arterial branch with oxygenated normal Tyrode’s solution composed of (in mmol/l) 129 NaCl, 20.0 NaHCO\(_3\), 0.5 MgSO\(_4\), 4.0 KCl, 5.5 dextrose, 0.9 NaH\(_2\)PO\(_4\), and 1.8 CaCl\(_2\). Perfusion pressure was maintained at 40 mmHg (which remained constant at 1,000 ms. Isochrone interval throughout the entire protocol, the pacing cycle length (CL) of (in mm) 14 mm, and a 0.95-mm spacing between recording sites.

**Ventricular electrical remodeling protocol.** The following remodeling protocol was performed in 19 preparations (13 LV, 6 RV): the endocardial surface of the canine wedge preparation was stimulated at 1.2 times diastolic threshold current using a polytetrafluoroethylene-coated silver unipolar electrode (0.1-mm diameter). The pacing protocol is illustrated in Fig. 1, B–D. To ensure steady-state conditions, the preparation was stimulated from the endocardial surface at a cycle length of 1,000 ms until a constant QT interval (measured from the
ECG) was observed for at least 30 min (preremodeling phase). Once a steady-state condition was achieved, the sequence of transmural propagation was reversed by stimulation of the epicardium at the same cycle length for 40 min (remodeling phase). After 40 min of epicardial pacing, the original activation sequence was restored by resuming endocardial pacing at a cycle length of 1,000 ms for an additional 30–40 min (postremodeling phase). Control measurements were obtained in five preparations (3 LV, 2 RV) where the identical protocol was performed except that pacing site was not changed, and the preparations were stimulated from the endocardial surface at a cycle length of 1,000 ms during the entire protocol.

Optical action potentials were measured simultaneously from 256 sites on the epicardial surface every 10 min. In the first 10 min of the remodeling and postremodeling phases, additional recordings were made every 1 to 2 min. To measure the effect of electrical remodeling, action potential properties were compared during the pre- and post-remodeling phases. In this way, each preparation served as its own control. In eight additional preparations (7 LV, 1 RV), action potentials were recorded from the transmural surface to examine remodeling of cell types across the transmural wall.

Single-cell recordings. To determine whether changes in action potential notch observed in optical action potential maps was specifically attributable to remodeling of Ih, epicardial myocytes were isolated from the LV epicardium (16 myocytes) using a standard enzymatic dispersion technique. The canine wedge preparation was perfused with an enzyme solution containing collagenase II (1.5 mg/ml) for 50 min at −5 °C/min. A 2-mm layer was excised from the epicardial surface and placed in a fresh collagenase solution at 35°C and agitated gently for 5–10 min. The filtrates were washed twice in 5 ml of Tyrode’s solution containing 5 mg/ml bovine serum albumin and 1 mmol/l CaCl2. The isolated myocytes were removed from solution and resuspended in 10 ml of Dulbecco’s modified Eagle’s medium.

Single-cell action potentials were recorded using the perforated patch method (32). Microelectrodes were fabricated from TW150F borosilicate glass capillaries, filled with a solution composed of (in mmol/l) 120 aspartic acid, 20 KCl, 2 MgCl2, and 5 HEPES, and brought to a pH of 7.3. Nystatin was dissolved in dimethyl sulfoxide in a 400-ms voltage step ranging from −30 to +60 mV for 400 ms at a cycle length of 1,000 ms. This waveform, therefore, simulated the epicardial action potential amplitude measured during epicardial pacing in the wedge preparation.

The second protocol used a high-amplitude voltage clamp (simulated low electrotonic load, n = 7). Enhanced upstroke amplitude was simulated by 40 min of repetitive high-amplitude voltage pulses from a holding potential of −70 mV to +60 mV for 400 ms at a cycle length of 1,000 ms. This waveform, therefore, simulated the epicardial action potential amplitude measured during endocardial pacing in the wedge preparation.

The third protocol did not use a voltage clamp (simulated normal electrotonic load, n = 5). Normal upstroke amplitude was achieved by eliciting action potentials at a cycle length of 1,000 ms for 40 min, without altering action potential amplitude with either high-or low-amplitude voltage pulses.

Data analysis. APD was measured using an automated computer algorithm as the difference between depolarization and repolarization times (10, 17, 18). Depolarization time was defined as the point of maximum positive derivative of the action potential, and repolarization time was defined as the point of maximum positive second derivative of the repolarization phase, as described previously. Each activation and repolarization time was inspected, and accuracy was verified by the investigators. Electrotetnic loading during propagation was assessed from optically recorded action potential upstroke amplitude and slope. Action potential upstroke amplitude was defined as the difference between the resting membrane potential and the peak amplitude of the upstroke (see Fig. 4, inset). Similarly, optically recorded action potential notch amplitude and slope were used as indexes of Ih activity. For the purpose of this study, notch amplitude was used to refer to phase 1 magnitude, as previously described (20, 21). Notch amplitude was defined as the difference between the maximum upstroke amplitude and the local minimum of the notch. To allow comparisons of relative amplitude between recording sites, the upstroke and notch amplitudes at each site were normalized to the steady-state upstroke amplitude at that site as validated previously (22). Although the absolute upstroke amplitude cannot be determined by using our methods, normalization was performed to allow time-dependent changes in amplitude to be compared between recording sites.

Optical mapping can produce minor conduction velocity-dependent temporal blurring in action potential upstroke due to spatial averaging within each recording pixel (10). Therefore, a computer model was used to simulate the effect of spatial averaging due to a change in activation sequence, which confirmed that the observed activation sequence-dependent changes in upstroke amplitude cannot be attributed to a blurring artifact.

Statistical analysis. Changes in APD, upstroke amplitude, and notch amplitude from control and electrically remodeled wedges were compared using a paired Student’s t-test. Bonferroni’s correction was applied for multiple comparisons where appropriate. P < 0.05 was considered statistically significant.

RESULTS

Activation sequence-dependent remodeling of action potential. Action potential properties were measured from epicardial cells before and after a period of altered activation sequence. Remodeling was defined as a significant change from baseline in action potential properties that persisted when baseline activation sequence was restored. The effect of activation sequence on APD is shown in Fig. 2A. Mean APD recorded from the epicardial surface of nine wedge preparations is plotted during the three phases of the remodeling protocol: at the completion of baseline endocardial pacing (preremodeling), at the onset of the remodeling phase (after a change from endocardial to epicardial stimulation), and at the onset of the postremodeling phase (after a change from epicardial to endo-

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cardial stimulation). When transmural propagation was changed (i.e., during the remodeling phase), APD shortened by 21.0 ± 9.8 ms. When endocardial pacing was restored (postremodeling phase), APD is persistently prolonged compared with the preremodeling phase. B: upstroke and notch amplitudes measured from the same epicardial sites in nine preparations during the preremodeling, remodeling, and postremodeling phases. Note that activation sequence-dependent changes in activation in upstroke amplitude are closely tracked by changes in notch amplitude. However, changes in upstroke amplitude are completely reversible, whereas changes in notch amplitude are not (i.e., notch amplitude is remodeled).

The effect of altered activation sequence on epicardial action potential upstroke amplitude and notch amplitude is also shown in Fig. 2B. Changing from endocardial to epicardial pacing resulted in significant attenuation of both action potential upstroke amplitude (by 13.9 ± 3.8%; P < 0.001) and notch amplitude (by 73.8 ± 7.6%; P < 0.0001) in epicardial cells. When endocardial pacing was restored, upstroke amplitude immediately returned to baseline levels. In contrast, the notch amplitude did not recover, and remained attenuated by 23.4 ± 10.0% (P < 0.0005). In five wedge preparations that did not undergo a change in activation sequence over identical time periods of observation (controls), there was no significant (NS) change in APD (173.8 ± 2.9 ms vs. 173.0 ± 1.3 ms, P = NS), upstroke amplitude (100.0 ± 2.3% vs. 100.4 ± 2.4%, P = NS) or notch amplitude (100.0 ± 3.7% vs. 96.7 ± 2.1%, P = NS) over an identical time period. These results were further supported by measurements of the slope of the action potential upstroke and notch. As shown in Fig. 3, a change in activation sequence significantly attenuated the slope of both the upstroke and notch. When baseline activation was restored (postremodeling phase), the slope of the upstroke immediately recovered to baseline levels. However, the slope of the notch did not fully recover, and remained significantly attenuated (P < 0.025). Taken together, these data indicate that the action potential notch is remodeled by a relatively short period of altered activation sequence while the upstroke amplitude is not.

Fig. 2. Electrical remodeling of epicardial action potential duration (APD; A) and upstroke (B) and notch (C) amplitudes. A: mean epicardial APD from nine wedge preparations during the three phases of the remodeling protocol. During the remodeling phase, APD moderately shortens. When baseline pacing is restored (postremodeling phase), APD is persistently prolonged compared with the preremodeling phase. B: upstroke and notch amplitudes measured from the same epicardial sites in nine preparations during the preremodeling, remodeling, and postremodeling phases. Note that activation sequence-dependent changes in activation in upstroke amplitude are closely tracked by changes in notch amplitude. However, changes in upstroke amplitude are completely reversible, whereas changes in notch amplitude are not (i.e., notch amplitude is remodeled).

Fig. 3. Electrical remodeling of the slope of action potential upstroke (A) and notch (B). The slope (best-fit linear regression) of the action potential upstroke and notch was measured from multiple epicardial sites during the preremodeling, remodeling, and postremodeling phases. A change in activation sequence (during the remodeling phase) attenuated the slope of both the upstroke and notch. When baseline activation was restored (postremodeling phase), the slope of the upstroke immediately recovered to baseline levels. However, the notch did not fully recover, and remained significantly attenuated. NS, not significant.
Figure 4 demonstrates the relationship between action potential upstroke and notch amplitudes during the three phases of the remodeling response in a representative experiment. In this example, upstroke and notch amplitudes were recorded simultaneously and averaged from eight sites on the epicardial surface, and are plotted during the three phases of the remodeling protocol. Immediately after the change from endocardial to epicardial stimulation, the upstroke amplitude in epicardial cells decreased by 14.9 $\pm$ 2.4% ($P < 0.001$). However, during 40 min of continued epicardial pacing, upstroke amplitude remained essentially constant, and did not decrease further. After restoration of endocardial pacing, the upstroke amplitude immediately returned to baseline levels, indicating again that activation sequence-dependent changes in upstroke amplitude were more likely related to passive electrical properties of the tissue rather than remodeling of ionic currents. Notch amplitude of epicardial cells was also significantly attenuated (by $73.0 \pm 7.7\%$, $P < 0.0001$) immediately after a change from endocardial to epicardial stimulation. However, in contrast to upstroke amplitude, notch amplitude continued to diminish (by $5.2 \pm 1.5\%$, $P < 0.0001$) during the period of epicardial pacing, suggesting progressive remodeling of $I_{\text{to}}$. After restoration of endocardial pacing, the upstroke amplitude did not recover completely to baseline levels, and remained attenuated by $25.6 \pm 8.1\%$ ($P < 0.00025$), also consistent with remodeling of $I_{\text{to}}$. The very close correspondence between changes in upstroke and notch amplitude suggests that reduced upstroke amplitude attenuated $I_{\text{to}}$ (which primarily forms the action potential notch) according to its known voltage-dependent properties, triggering electrical remodeling. These results were observed in preparations isolated from both the LV and RV.

The example in Fig. 4 shows a parallel fluctuation in both upstroke and notch amplitude during the preremodeling phase. However, in a systematic analysis of all preparations, this variability did not reach statistical significance, in contrast to the statistically significant changes induced by the remodeling phase.

**Voltage-dependent remodeling of $I_{\text{to}}$.** To determine whether the period of reduced upstroke amplitude during epicardial stimulation was indeed responsible for remodeling of $I_{\text{to}}$, notch amplitude and $I_{\text{to}}$ density were measured from isolated epicardial myocytes subjected to voltage-clamp protocols designed to simulate periods of reduced upstroke amplitude, enhanced upstroke amplitude, and normal upstroke amplitude. Notch amplitude and $I_{\text{to}}$ density were compared before and after the period of simulated altered upstroke amplitude. As shown in Fig. 5, myocytes that underwent a 40-min period of low-amplitude voltage pulses experienced a significant reduction in notch amplitude (by $7.3 \pm 2.5\%$, $P < 0.025$). In contrast, myocytes that underwent a 40-min period of high-amplitude voltage pulses experienced a significant increase in notch amplitude (by $19.2 \pm 4.7\%$, $P < 0.005$). Control myocytes, in which action potentials were stimulated for an equivalent time period without high-or low-amplitude voltage pulses, did not exhibit any changes in notch amplitude ($52.6 \pm 7.4$ vs. $52.8 \pm 6.7$ mV). These data demonstrate that a brief (40 min) period of altered voltage can trigger persistent changes in notch amplitude, as was observed in wedge preparation experiments. In contrast, myocytes in all three groups did not exhibit a significant change in upstroke amplitude over the equivalent time period (low amplitude: $127.5 \pm 1.0$ vs. $126.2 \pm 1.5$ mV; high amplitude: $124.8 \pm 3.4$ vs. $133.4 \pm 5.0$ mV; control: $135.4 \pm 2.8$ vs. $135.8 \pm 2.0$ mV), providing further evidence for notch remodeling in the absence of upstroke remodeling.

Figure 6A illustrates that remodeling of $I_{\text{to}}$ was responsible for the observed changes in notch amplitude. Myocytes that underwent a 40-min period of low-amplitude voltage pulses exhibited a reduced peak $I_{\text{to}}$ (by $12 \pm 4\%$) in the postremodeling phase compared with the preremodeling phase. In contrast, myocytes that underwent a 40-min period of high-amplitude voltage pulses exhibited a significant increase in $I_{\text{to}}$ in the postremodeling phase (by $60 \pm 20\%$, $P < 0.01$). The current-voltage relationship (Fig. 6B) shows the voltage-dependent increase in $I_{\text{to}}$ density caused by the remodeling period of high-amplitude voltage pulses.

**Activation sequence-dependent electrotonic loading alters upstroke amplitude.** From these experiments, it was apparent that activation sequence-dependent changes in upstroke amplitude triggered remodeling of $I_{\text{to}}$. However, the mechanism responsible for changing upstroke amplitude in response to a change in activation sequence is unknown. We hypothesized that a change in electrotonic load associated with a change in activation sequence was responsible for attenuating upstroke...
amplitude, which, in turn, triggered electrical remodeling of $I_{Na}$. To test this hypothesis, upstroke amplitude was measured from epicardial action potentials during epicardial pacing in the wedge preparation. As shown in Fig. 7 from a representative experiment, action potentials were selected along a line originating at the site of pacing. The extent of electrotonic remodeling was highly dependent on the distance from the site of stimulation. Proximal to the site of epicardial pacing, upstroke amplitude was maximally attenuated (by 24.4%). Distal to the site of epicardial pacing, upstroke amplitude attenuation decreased linearly at a rate of 5.9%/mm. As expected, the effect of electrotonic loading on action potential upstroke amplitude was not dependent on the ionic composition of the cells (epicardial, midmyocardial, or endocardial) or the location of the preparation (RV or LV). As shown in Fig. 8A, when stimulating from midmyocardial layers of cells, we observed activation sequence-dependent reduction in notch amplitude. Sites selected from the same transmural depth, which are expected to have similar action potential properties, had significantly different notch amplitudes. At site A (close to the site of pacing), there was no discernible phase 1 notch. At recording sites (sites B–D) further away from the pacing site, the notch amplitude progressively increases. Importantly, when the activation sequence was reversed (Fig. 8B), the pattern of notch attenuation also reversed.

To further determine the importance of electrotonic load on the action potential upstroke of cardiac myocytes, a field stimulus (50 V, 1.0 ms) was delivered from two metal plates in the imaging chamber, to measure action potential upstroke amplitude properties in the absence of electrotonic loading normally imposed by propagation. Nonpropagating (i.e., unloaded) upstroke amplitude was measured from 256 transmural sites and was compared with upstroke amplitude measured during point (i.e., loaded) stimulation. By eliminating the electronic load at all sites, action potential upstroke amplitude increased by $54.1 \pm 7.2\%$ during field stimulation compared with point stimulation ($P < 0.0001$). Therefore, propagation, and hence activation sequence, imposes an electrotonic load that acts to attenuate action potential upstroke amplitude proximal to the site of stimulation.

**DISCUSSION**

In the ventricle, electrical remodeling has been classically associated with changes in T-wave morphology (i.e., T-wave memory) (24). Interestingly, in contrast to atrial remodeling, ventricular remodeling is associated with prolongation of repolarization (9, 15, 25–27, 35). Rapid ventricular pacing for periods lasting as short as 1 h can cause persistent changes in ventricular repolarization (6, 26, 27, 35). Ventricular remodeling produced by a change in activation sequence (i.e., ventricular pacing) over 3 wk in the dog was associated with reduced expression of $I_{Na}$ and $I_{Na}$ channel mRNA (35). Moreover, $I_{Na}$ remodeling was inhibited by the protein synthesis inhibitor cycloheximide, suggesting that ventricular remodeling may be caused by changes in expression of sarcolemmal currents (27). However, a major unresolved question is how a change in activation sequence actually triggers a change in expression of ion currents. Why would voltage and time-dependent ion channels within the cell membrane be affected by the direction by which the myocyte receives depolarizing current?

In this study, we found that a change in activation sequence significantly alters the pattern of electrotonic load on myocardium. Cells close to the site of pacing experience the greatest electrotonic load and therefore have the maximum attenuation of action potential upstroke amplitude (Fig. 7). When electrotonic load due to propagation was reduced, such as with field stimulation, upstroke amplitude was markedly enhanced. Because significant changes in upstroke amplitude occurred simultaneously with altered activation sequence, these changes cannot be explained by altered cellular excitability. Instead, altered upstroke amplitude is likely due to the mass of downstream repolarized cells, which electrotonically reduces the upstroke amplitude of upstream cells. Although altered electrotonic loading can also be caused by increased curvature or pivoting of propagating wavefronts (4, 11) or by tissue geom-
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Fig. 6. Voltage-dependent electrical remodeling of $I_{\text{to}}$. Peak $I_{\text{to}}$ current was measured during a voltage step protocol (inset) during the preremodeling and postremodeling phases of the remodeling protocol due to 40 min of simulated reduced upstroke amplitude (low-amplitude voltage pulses) and simulated enhanced upstroke amplitude (high-amplitude voltage pulses). A: myocytes that underwent a 40-min period of reduced upstroke amplitude experienced a significant decrease in $I_{\text{to}}$ in the postremodeling phase. The current-voltage relationship shows a voltage-dependent decrease in $I_{\text{to}}$ density due to reduced upstroke amplitude ($^{*}P < 0.05$). B: in contrast, myocytes that underwent a 40-min period of enhanced upstroke amplitude experienced an increase in $I_{\text{to}}$ density in the postremodeling phase compared with the preremodeling phase. The current-voltage relationship shows a voltage-dependent increase in $I_{\text{to}}$ density due to reduced upstroke amplitude ($^{*}P < 0.05$).

Our data also suggest that electrotonic load-induced changes in action potential upstroke amplitude can trigger remodeling of $I_{\text{to}}$ on a surprisingly short time scale. When the activation pattern was altered (Figs. 2–4), an activation sequence-dependent reduction in upstroke amplitude was closely associated with significant reduction in notch amplitude and slope, likely due to the known voltage-dependent gating properties of $I_{\text{to}}$. While the upstroke amplitude remained constant during altered transmural activation, notch amplitude progressively decreased, consistent with a remodeling effect. The mechanism by which continued exposure to reduced action potential amplitude led to progressive reduction in $I_{\text{to}}$ current cannot be ascertained from our data. Presumably, reduced $I_{\text{to}}$ conductance induced by lower action potential amplitude attenuated $I_{\text{to}}$ according to its known voltage dependence, and may have triggered cell-signaling mechanisms that resulted in reduced current expression or activity. When normal activation was restored, upstroke amplitude returned immediately and completely to baseline, reaffirming that observed changes in notch and upstroke amplitude cannot possibly be attributed to artifacts related to photobleaching in a voltage-sensitive dye. In contrast to upstroke amplitude, notch amplitude remained persistently attenuated, providing further evidence for $I_{\text{to}}$ remodeling.

These results were confirmed in recordings made from isolated epicardial myocytes, where a 40-min period of low-amplitude voltage clamps persistently attenuated notch amplitude and downregulated $I_{\text{to}}$ (Figs. 5 and 6). These results are consistent with previously reports of reduced $I_{\text{to}}$ density and decreased Kv4.3 mRNA level in ventricular myocytes from electrically remodeled hearts (9, 35). Although we found changes in $I_{\text{to}}$ on a much shorter time scale than those reported previously, there is evidence that ventricular electrical remodeling can be induced in as little as 1 h (6, 25, 26) and changes in the mRNA level of voltage-dependent potassium channels has been reported in as little as 30 min (34). However, because we employed transmural optical mapping in our study, we were able to identify, for the first time, a potential mechanism responsible for triggering activation sequence-dependent remodeling of $I_{\text{to}}$.

Clearly, the isolated myocyte recordings were not made under conditions that exactly replicate those in the intact tissue. It is a recognized and accepted limitation that the process of dissociating and impaling myocytes can potentially alter the activity of some ion channels. In addition, although voltage pulse amplitude differences used in these studies (15–30%) approximated those observed experimentally, we did not at-
how or if remodeling of \( I_{ox} \) may induce persistent changes in APD, except to say that APD prolongation was associated with \( I_{ox} \) remodeling. However, other investigators have demonstrated that electrical remodeling can be attenuated by blocking \( I_{ox} \) current, providing evidence for the important role of \( I_{ox} \) in electrical remodeling of ventricular repolarization (9).

An alternative, but not mutually exclusive, mechanism for triggering electrical remodeling is related to activation sequence-dependent alterations in regional wall stress. Altered activation sequence is known to cause regional differences in contractile work and produce inhomogeneous hypertrophy in canine ventricle (23). It is conceivable that these changes can induce electrical remodeling in the ventricle via mechanical-electrical feedback mechanisms. However, it is unlikely that these mechanisms were operative in our experiments, as the wedge preparations were not exposed to mechanical loading.

APD is modulated by a complex and delicate balance of inward and outward currents. Even small changes in notch amplitude can modulate voltage-dependent activation of inward calcium and outward potassium current, which can influence APD (13). However, one cannot conclude from our data that \( I_{ox} \) is the only ion current that is affected by altered activation sequence. In fact, the significant and persistent change in APD shown by other investigators may imply that other currents are also being remodeled (26). However, because of the unique fingerprint of \( I_{ox} \) on the action potential (the phase 1 notch), its time- and sequence-dependent changes are clearly visible. Further work is needed to determine the role of \( I_{ox} \) in remodeling-induced changes in APD.

Although we focused on altered activation sequence in ventricle, these data suggest the intriguing hypothesis that altered electrotonic load may also be the trigger of electrical remodeling in atria, particularly during atrial fibrillation. Finally, although remodeling is arrhythmogenic in atria (33) and there is some evidence of enhanced susceptibility to ventricular...
arrhythmias after remodeling in the ventricle (26), further work is needed to establish the effect of electrical remodeling on susceptibility to ventricular arrhythmias.

GRANTS

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