Action potential characterization in intact mouse heart: steady-state cycle length dependence and electrical restitution

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Knollmann BC, Schober T, Petersen AO, Sireno SG, Franz MR. Action potential characterization in intact mouse heart: steady-state cycle length dependence and electrical restitution. Am J Physiol Heart Circ Physiol 292: H614–H621, 2007. First published September 8, 2006; doi:10.1152/ajpheart.01085.2005.—Transgenic mice have been increasingly utilized to investigate the molecular mechanisms of cardiac arrhythmias, yet the rate dependence of the murine action potential duration and the electrical restitution curve (ERC) remain undefined. In the present study, 21 isolated, Langendorff-perfused, and atrioventricular node-ablated mouse hearts were studied. Left ventricular and left atrial action potentials were recorded using a validated miniaturized monophasic action potential probe. Murine action potentials (AP) were measured at 30, 50, 70, and 90% repolarization (APD_{30}–APD_{90}) during steady-state pacing and varied coupling intervals to determine ERCs. Murine APD showed rate adaptation as well as restitution properties. The ERC time course differed dramatically between early and late repolarization: APD_{30} shortened with increasing S1–S2 intervals, whereas APD_{90} was prolonged. When fitted with a monoeponential function, APD_{30} reached plateau values significantly faster than APD_{90} (τ = 29 ± 2 ms vs. 78 ± 6 ms, P < 0.01, n = 12). The slope of early APD_{90} restitution was significantly <1 (0.16 ± 0.02). Atrial myocardium had shorter final repolarization and significantly faster ERCs that were shifted leftward compared with ventricular myocardium. Recovery kinetics of intracellular Ca^{2+} transients recorded from isolated ventricular myocytes at 37°C (τ = 93 ± 4 ms, n = 18) resembled the APD_{90} ERC kinetics. We conclude that mouse myocardium shows AP cycle length dependence and electrical restitution properties that are surprisingly similar to those of larger mammals and humans.

METHODS

Isolated Perfused Heart Preparation

Twenty-one mice were anesthetized via intraperitoneal injection of 0.5 ml of tribromoethanol solution (2.5% vol/vol). Hearts were removed under surgical plane of anesthesia and perfused with Krebs-Henseleit buffer (containing in mM: 118 NaCl, 4.7 KCl, 1.2 NaH_{2}PO_{4}, 25 NaHCO_{3}, 2.5 CaCl_{2}, 1.2 MgCl_{2}, 11 glucose, and 0.5 EDTA) as described previously (18). Studies were approved by the Institutional Animal Care and Use Committee and were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Monophasic Action Potential Recordings

Atrial and ventricular monophasic action potentials (MAP) were recorded from isolated hearts using a validated microprobe (18, 19). This probe could be held perpendicular at a single ventricular or atrial epicardial site or moved from one site to another as required. The previously described (19) horizontally placed Langendorff setup allowed stable recordings with the miniaturized contact MAP probe from the left atrial (LA) or left ventricular (LV) epicardial heart surface (see Fig. 1).

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Ventricular Pacing Protocol

After thermal ablation of the atrioventricular node, a platinum pacing electrode was inserted in the LV free wall in close proximity to the MAP recording electrode, and ventricular pacing at twice threshold was begun. Pacing protocols were generated using a custom-built programmable stimulator generously provided by Scott Lamp and Dr. James Weiss, University of California, Los Angeles Cardiovascular Research Laboratory. The ERC is defined as the time course with which APD (at a defined repolarization level) recovers from its shortest duration at the most premature extra beat to its longest duration at the maximal achievable interval (escape interval). To obtain the ERC, we paced each heart with repeated trains of 30–40 beats (S1), followed by a single S2 with consecutively shorter S1–S2 coupling intervals. The basic cycle lengths of the S1 trains were 100, 150, and 300 ms. Effective refractory period was determined as the first S1–S2 coupling interval that failed to produce a ventricular response. To determine the steady-state APD-cycle length relationship, we paced hearts with trains of 40 beats starting at a cycle length of 400 ms. Paced cycle length was successively shortened until refractoriness was reached. To construct the steady-state APD-cycle length relationship (see Fig. 3C), we analyzed only the last three beats of each train.

Atrial Pacing Protocol

A platinum pacing electrode was inserted in the atrial appendage of the left atrium in close proximity to the MAP recording electrode, and ventricular pacing at twice threshold was begun. All ERCs were determined at a basic cycle length of 130 ms. Because of the high intrinsic rate of the atria (~140–150 ms cycle length), no steady-state APD-cycle length relationship was recorded.

APD Analysis

APDs were determined at different repolarization levels (APD90, APD50, APD10, and APD50), with full repolarization defined as 100% and the tip of the MAP upstroke as 0% (see Fig. 2B). Time of maximum upstroke velocity (dV/dt max) was defined as time 0. Full repolarization was defined at 10 ms immediately preceding the onset of the MAP upstroke. For the analysis of very early premature responses (S2) inscribed on the terminal repolarization of the last S1 beat, full repolarization was taken to be the same as for the preceding S1 beat. Only stable MAP recordings with straight diastolic potentials and void of movement artifacts were used for analysis.

S1–S2 interval was determined as the time from dV/dt max of the last beat of the pacing train (S1) to the dV/dt max of the S2 beat. During short S1–S2 coupling intervals, the measured S1–S2 interval was longer than the time between the S1–S2 pacing stimulus as a result of local latency (i.e., the time from pacing spike to dV/dt max of the AP, see Fig. 2B). Therefore, in accordance with previous MAP studies (12), actual upstroke intervals rather than paced S1–S2 intervals were used to construct the ERC.

Intracellular [Ca2+] Measurements in Isolated Ventricular Myocytes

Single myocytes were isolated by enzymatic digestion as previously described (20). Myocytes were then loaded with the Ca2+-sensitive dye by a 20-min incubation with 5 μM fluo-3 AM (ace-toxymethyl ester form; Molecular Probes) at room temperature in Tyrode buffer (1.2 mM Ca2+) and then washed two times for 10 min each with normal Tyrode buffer (containing in mM: 134 NaCl, 5.4 KCl, 2 CaCl2, 1 MgCl2, 10 glucose, and 10 HEPES, pH 7.4 adjusted with NaOH) in the dark to allow for deesterification of the fluo-3 AM. Ca2+ transients were evoked by electrical field stimulation at 37°C in the normal Tyrode solution. Fluorescence signals of fluo-3 were detected by a fluorescence system interface (IonOptix). The fluo-3-loaded cells were excited at 488 nm with a xenon arc lamp, and the emitted fluorescence was measured at a wavelength of 515 nm by using a photomultiplier attached to an inverted microscope. Whole cell Ca2+ transients were constructed by recording the fluorescent signal from the entire cell with a sampling rate of 1 kHz. Changes of intracellular [Ca2+] were represented by the normalized fluorescence F/F0, where F is fluorescence intensity and F0 is resting fluorescence measured immediately before the last S1 stimulus for each train. The restitution curve of Ca2+ transients was constructed by applying repeated trains of 20 stimuli (S1) at 2 Hz, followed by an extra stimulus (S2) at varying coupling intervals after the last S1. Stimulus duration was 5 ms at twice the pacing threshold.

Statistical Evaluation

Groups were compared by using ANOVA followed by post hoc Student’s t-test whenever applicable. Results are reported as means ± SE unless otherwise indicated.

RESULTS

Steady-State APD

Figure 1 depicts a typical murine MAP recording from the LV epicardial surface at a paced cycle length of 150 ms. The MAP was characterized by a sharp upstroke, followed by a quick repolarization to ~50–60% of its total amplitude, assumed a slower repolarization time course producing a “shoulder” or “low plateau,” and then gradually returned to the diastolic potential.

Restitution of Murine APD

Figure 2A shows a steady-state ventricular murine MAP recorded at a cycle length of 150 ms with superimposed premature responses at successively longer S1–S2 coupling intervals. The superimposed MAPs illustrate the recovery time course (restitution) of successively longer coupled responses. The most premature MAP had a smaller amplitude and significantly shorter duration than the basic MAP. With increasing
S1–S2 coupling intervals, MAP amplitude recovered and APD$_{90}$ lengthened. Interestingly, early repolarization (APD$_{30}$) of the very premature S2 was longer compared with the basic AP.

Figure 2B compares the time course of APD restitution at 30 and 90% repolarization. The ventricular electrical restitution curve differed dramatically between early and late repolarization: APD$_{30}$ restitution was inverse to APD$_{90}$ restitution, showing a negative initial slope. APD$_{30}$ shortened with increasing S1–S2 intervals, whereas APD$_{90}$ was prolonged. When fitted with a monoexponential function, APD$_{30}$ reached plateau values significantly faster than APD$_{90}$ ($\tau = 27 \pm 2$ vs. $78 \pm 6$ ms, $P < 0.01, n = 12$). The initial rapid lengthening of APD$_{90}$ at short coupling intervals also can be approximated by a straight line and has been implicated as a mechanism for arrhythmogenesis (32). The slope of this early restitution (coupling intervals of <150 ms) for APD$_{90}$ was significantly $<1$ ($0.16 \pm 0.02, n = 12$; see also Figs. 2C and 3).

**Cardiac Rate Memory**

The effects of steady-state pacing versus single cycle length alterations (ERC) are compared in Fig. 3. Figure 3, A and B, compares APD$_{90}$ as a function of restitution (S1–S2) versus APD$_{90}$ as a function of steady-state cycle length. The steady-state APD$_{90}$ was significantly shorter than the ERC-APD$_{90}$ at longer pacing cycle lengths (>200 ms, Fig. 3A). There were no significant differences at shorter cycle length (Fig. 3B) despite an apparent crossing over of the restitution curves. Figure 3C compares APD$_{90}$ restitution recorded at two different steady-state cycle lengths. The ERC after fast pacing trains (100-ms cycle length) was shifted toward shorter APD values compared with the ERC after slow pacing trains (300-ms cycle length). Together, these results demonstrate that sustained pacing caused additional rate adaptation compared with single cycle length alterations.

**Atrial vs. Ventricular APD and Their Restitution**

Table 1 compares MAP parameter values from LA and LV epicardial recordings obtained at a steady-state pacing cycle length of 130 ms. Atrial MAPs were significantly shorter than those recorded from ventricle. This difference was due to a shorter final repolarization phase (APD$_{70}$, APD$_{90}$). The atrial murine MAP lacked the shoulder, or late plateau, of the ventricular MAP (Fig. 4, A–C). In contrast, average APD$_{30}$ was

![Fig. 2. Electrical restitution of murine ventricular AP.](image-url)
significantly longer in the left atrium compared with the left ventricle (Table 1).

Figure 4, D and E, shows representative examples of APD$_{30}$ and APD$_{90}$ restitution measured in the left ventricle and left atrium of the same heart. Overall, atrial restitution curves had shapes similar to those recorded from ventricular tissue. Because of the shorter atrial APD$_{70}$ and resulting shorter effective refractory period, the atrial ERCs were shifted to the left compared with the ventricular ERCs (Fig. 4, D and E). Furthermore, the atrial ERCs restituted significantly faster, reaching plateau values earlier than the ventricular ERC. Figure 5 compares the respective restitution rate constants of atrial and ventricular ERCs.

**Restitution of Intracellular Ca$^{2+}$/H$^{+}$ Transients**

As shown in Fig. 1, the final repolarization phase of the murine AP is relatively drawn out, forming a low plateau. Previous work in isolated ventricular myocytes demonstrated that this low plateau of the murine AP is produced by the inward current generated by the Na$^+$/Ca$^{2+}$/H$^{+}$ exchanger during electrogenic Ca$^{2+}$ extrusion of Ca$^{2+}$ released with each beat (20). Thus we next examined the restitution properties of intracellular Ca$^{2+}$/H$^{+}$ transients in ventricular myocytes. As illustrated in Fig. 6, Ca$^{2+}$/H$^{+}$ transients recovered in exponential fashion with longer S1–S2 coupling intervals. When fitted with a monoexponential function, Ca$^{2+}$/H$^{+}$ transients restituted with an average $\tau$ of 93 ± 4 ms ($n$ = 18). Hence, the isolated cell restitution kinetics of Ca$^{2+}$/H$^{+}$ transients were similar to those of the APD$_{90}$ ERC recorded in the isolated heart ($\tau$ = 78 ± 6 ms, Fig. 5B).

**DISCUSSION**

Mice have ECG characteristics very different from humans and repolarization of mouse ventricular myocardium is not readily derived from the mouse QT interval (24). This study is the first to describe the ventricular and atrial AP, its steady-state cycle length dependence, and its restitution kinetics in the isolated, perfused mouse heart.

**Differences in AP Characteristics Between Mice and Other Mammalian Species**

**AP wave shape.** The mouse AP shape differs from that of most other mammalian species (e.g., cat, dog, guinea pig, pig, and human) by the absence of an early, “high” plateau phase (see Fig. 1). The lack of a high plateau has been attributed to the predominance of rapidly activating outward K$^+$ currents encoded by several different K channel genes (Kv1.4, 1.5, 2.1, 4.2/4.3; reviewed in Ref. 26). In particular, the fast transient outward current produced by the heteromultimeric assembly of Kv4.2 and Kv4.3$\alpha$-subunits (15) is responsible for >50% of total outward K$^+$ currents in LV epicardial myocytes (7), resulting in very short APD$_{30}$ and APD$_{50}$ values. Consistent with these data, the Kv4.2/4.3 current density of LV epicardial...
myocytes is twice that of endocardial myocytes, resulting in endocardial APD\textsubscript{50} values that are twice as long as those of epicardial myocardium (7, 18). Interestingly, the overall action potential duration (APD\textsubscript{90}) of murine epicardium and endocardium is the same in isolated myocytes (7) or intact beating hearts (18). This suggests that despite its large current density, the Kv4.2/4.3 current contributes little to the terminal action potential duration of the mouse heart, as recently also demonstrated in the Kv4.2 knockout mouse model (15).

The final repolarization phase of the murine AP is relatively more drawn out, forming a “low” plateau phase at approximately \(-40\) mV (18). Given such a negative membrane potential, neither the Kv4.2/4.3 currents nor the L-type Ca\textsuperscript{2+} current are likely to contribute much to the low plateau phase (both channels gate at more positive membrane potentials and are largely inactivated at \(-40\) mV; see also Ref. 27). Rather, the low plateau is produced by the inward current generated by the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger during the electrogenic Ca\textsuperscript{2+} extrusion of the Ca\textsuperscript{2+} released during each beat, which previously has been demonstrated for the rat (10, 30) and mouse (20) action potential of ventricular myocytes. At the same time, the relatively small outward conductance of the inward rectifier current \(I_{K1}\) of mouse myocytes (17) allows even a modest Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange current to maintain the membrane potential at the low plateau so characteristic for the murine AP.

**APD restitution.** A striking feature of the murine electrical restitution curve is that early repolarization (APD\textsubscript{30}) rapidly shortened with longer coupling intervals, resulting in an ERC
that decayed with relatively fast kinetics (τ = 29 ms; see Figs. 3 and 5). This APD₃₀ shortening with longer S₁–S₂ coupling intervals can be the result of either reduced inward currents during early repolarization or the recovery of strong repolarizing currents. Both major inward currents (I_{Na} and I_{Ca}) increase with longer coupling intervals during the relative refractory period and therefore would tend to prolong the APD₃₀. In contrast, Kv4.2/4.3 currents recover rapidly from inactivation with a τ of 30 ms (33) and likely govern the shape of murine APD₉₀ ERC. The other major repolarizing K⁺ current (Kv1.5) of mouse ventricle restitutes with a very slow τ of 15 s (33) and thus is less likely to contribute much to APD₉₀ ERC.

Unlike the restitution of early repolarization, late repolarization (APD₉₀) was prolonged with longer coupling S₁–S₂ intervals (Fig. 2). The Na⁺/Ca²⁺ exchanger is primarily responsible for shaping the APD₉₀ of mouse and rat ventricular myocytes (10, 20, 30). Since the amount of exchanger current generated is directly proportional to the intracellular Ca²⁺ release during the twitch (27), the restitution of intracellular Ca²⁺ transients likely contributes importantly to the restitution properties of APD₉₀. Consistent with this idea, the time course of the S₂ Ca²⁺ transient restitution recorded from isolated myocytes (Fig. 6) closely matched that of the APD₉₀ ERC recorded from ventricular epicardium (Fig. 5). Similar findings have been reported for rat ventricular myocardium, which also demonstrated a monoexponentially increasing APD₉₀ with longer S₁–S₂ coupling intervals (16), as reported in the present study for mouse ventricular tissue (Figs. 2 and 3). Elimination of intracellular Ca²⁺ transients by application of BAPTA-AM abolished the low AP plateau and rendered the APD₉₀ ERC downsloping and similar to the APD₃₀ ERC, with successively shorter APD₉₀ values as S₁–S₂ coupling intervals became longer (16). Together, these data suggest that the restitution kinetics of Ca²⁺ release from intracellular stores determine the murine APD₉₀ ERC. Although technically difficult, simultaneous measurements of APs and Ca²⁺ transient restitution in intact contracting ventricular tissue are necessary to confirm this hypothesis.

Similarities in AP Characteristics Between Mice and Other Mammalian Species

**AP rate dependence.** Despite the above-mentioned ion channel differences between mice and human hearts, the steady-state relationship between cycle length and APD and the ERC time course were surprisingly similar by comparison. For the human ventricle, APD₉₀ increased from the fastest to slowest physiological heart rate range (cycle length 400 to 1,000 ms) and then reached a plateau (12). Likewise, the murine ventricular APD₉₀ increased from a cycle length of 80 to 150 ms (the physiological rate range for mice) and then remained flat with...
progressively longer cycle lengths. Opposite to what has been reported previously for the human myocardium (12), the mouse steady-state APD_{90} was shorter at longer cycle lengths than the S1–S2 restitution APD_{90} (see Fig. 3A). This divergence demonstrates that in the mouse ventricle the cumulative effect of a sustained cycle length change, sometimes also referred to as cardiac rate “memory” (31), is more than offset by the short-term effect of a rapid switch to a long S1–S2 interval. On the other hand, mouse ventricle did demonstrate a rate memory effect, since a faster priming rate (shorter steady-state cycle length) shifted the entire APD restitution curve downward (Fig. 3C), as also has been shown for canine (6) and human hearts (11). The downward shift reflects the lasting effect of a faster heart rate on the APD restitution curve. As recently reviewed by Carmeliet (8), the ion currents responsible for this phenomenon are subject to debate but likely involve, among others, an increase in Na\(^+/K\)\(^+\) pump current due to higher intracellular [Na\(^+\)] at fast pacing rates; faster reuptake of Ca\(^2+\) into sarcoplasmic reticulum, resulting in less inward current via the electrogenic Na\(^+/Ca\(^2+\) exchange; and activation of the delayed rectifier K\(^+\) currents (the latter is less likely to play a role in the mouse heart because of the lack of \(I_{Kf}\) in adult mouse ventricular myocytes; Ref. 25). In essence, the murine APD rate dependence and its recovery during individual test intervals are, for the most part, not unlike those of the human myocardium, only scaled to shorter cycle lengths.

Determining the ERC at a single repolarization level (commonly at 70–90%) lump together the restitution kinetics of several ion channels (13). We therefore defined murine APD restitution at two distinct repolarization levels. The APD_{90} ERC was upsloping, not unlike the ERC in humans (11), dogs (6), rats and ferrets (16), swine (4), and rabbits (11). However, the early slope of the murine APD_{90} restitution was much shallower than reported in other mammalian species; it was \(<0.2\). Thus it is less likely that restitution properties per se contribute to arrhythmogenesis in mice hearts, based on the restitution hypothesis that a slope of \(>1\) promotes wave break and ventricular fibrillation (32). The physiological heart rate of \(>500\) beats/min in mouse hearts would be ill served by that.

**Atrial versus ventricular APD.** In most mammalian species, the atrial AP is characterized by a shorter early repolarization phase, resulting in a triangular wave shape, compared with the ventricular AP with its usually pronounced plateau phase (22). We also found a triangular atrial AP wave shape in the mouse. However, unlike other mammals, the atrial mouse AP was longer during the early repolarization phase (APD_{30}) compared with that in the ventricle. This finding is likely the result of the 50% lower Kv4.2/4.3 current density found in atrial compared with left ventricular myocytes (23, 34).

This study recorded atrial APs from the intact beating mouse heart, for which there are no precedents. Previous work has examined murine atrial APs in isolated myocytes by using conventional whole cell current clamp (23, 34) or in isolated, superfused atrial preparations using optical mapping (28). In these studies, intracellular Ca\(^2+\) cycling and contraction was disrupted by either application of ryanodine (28) or intracellular dialysis with EGTA (34). Nevertheless, APD values recorded in both studies either from atrial tissue paced at a cycle length of 120 ms (APD_{70} of 15 ms) or from atrial myocytes (APD_{90} of 27 ms) were similar to APD values recorded from intact beating atria reported in the present study (see Table 1).

**Limitations**

The earliest premature responses had slower upstroke velocities. The duration of the upstroke was included in measurements of APD_{30} and may have exaggerated the high initial values of the APD_{30}-ERC. However, the APD_{30}-ERC also was found to have a negative initial slope in canine (6), cat (5), and rabbit myocardium (11). It also should be noted that significant electrical heterogeneity exists across the mouse heart, in both APD (18) and ionic currents (7). The data reported only represent the responses observed in the epicardial layer of the left atrium and ventricle. Restitution properties in other layers of the heart may be different.

In conclusion, despite significant divergence in AP shape between mouse myocardium and that of larger mammalian species, the fundamental relationship between cycle length and APD_{90} exists in the mouse heart, during both extrastimulation (ERC) and steady-state pacing, with the latter also producing rate memory effects. In the mouse ventricle, transient outward K\(^+\) currents appear to determine restitution properties of early repolarization, whereas intracellular Ca\(^2+\) dynamics likely shape restitution of late repolarization. These comprehensive data on the murine APD in response to sudden and sustained cycle length changes should be useful to scientists using the mouse heart and its many transgenic manipulations for modeling cardiac diseases, including arrhythmias.

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**REFERENCES**


34. Xu H, Li H, Nerbonne JM. Elimination of the transient outward current and action potential prolongation in mouse atrial myocytes expressing a dominant negative \( K\textsubscript{v}4 \) \( \alpha \) subunit. *J Physiol* 519: 11–21, 1999.