Heterogeneity of action potential durations in isolated mouse left and right atria recorded using voltage-sensitive dye mapping

Anders Nygren,1 Alan E. Lomax,1 and Wayne R. Giles1,2

1Department of Physiology and Biophysics, University of Calgary, Calgary, Alberta, Canada T2N 4N1; and 2Whitaker Institute of Biomedical Engineering, University of California, San Diego, La Jolla, California 92037-0412

Submitted 26 April 2004; accepted in final form 15 July 2004

Heterogeneity of action potential durations in isolated mouse left and right atria recorded using voltage-sensitive dye mapping. Am J Physiol Heart Circ Physiol 287: H2634–H2643, 2004. First published July 22, 2004; doi:10.1152/ajpheart.00380.2004.—An imaging system for di-4-ANEPPS (4-[N-(di-nbutylamino)-6-naphthaldehyde]pyridinium) voltage-sensitive dye recordings has been adapted for recording from an in vitro mouse heart preparation that consists of both atria in isolation. This approach has been used to study inter- and intra-atrial activation and conduction and to monitor action potential durations (APDs) in the left and right atrium. The findings from this study confirm some of our previous findings in isolated mouse atrial myocytes and demonstrate that many electrophysiological properties of mouse atria closely resemble those of larger mammals. Specifically, we made the following observations: 1) Activation in mouse atria originates in the sinusoidal atrium and spreads into the right atrium and, after a delay, into the left atrium. 2) APD in the left atrium is shorter than in the right atrium. 3) Sites in the posterior walls have longer APDs than sites in the atrial appendages. 4) Superfusion of this preparation with 4-aminoypyridine and tetraethylammonium resulted in increases in APD, consistent with their inhibitory effects on the K+ currents known to be expressed in mouse atria. 5) The muscarinic agonist carbachol shortened APD in all areas of the preparation, except the left atrial appendage, in which carbachol had no statistically significant effect on APD. These results validate a new approach for monitoring activation, conduction, and repolarization in mouse atria and demonstrate that the physiological and pharmacological properties of mouse atria are sufficiently similar to those of larger animals to warrant further studies using this preparation.

HETEROGENEITY OF THE REFRACTORY periods within or between mammalian atria has been implicated as a factor initiating and/or maintaining atrial rhythm disturbances (7, 28). A number of electrophysiological studies have identified significant regional differences in atrial refractoriness and/or action potential duration (APD) in canine (15, 16, 18, 24, 38) and rabbit (34, 45) atria. It is known that action potentials in the left atrium of the dog as well as the rabbit exhibit more rapid early repolarization and shorter APD than in the right atrium. Electrophysiological gradients have also been identified within each atrium. For example, within the canine right atrium, microelectrode recordings in intact tissue (18, 38) and whole cell patch recordings from isolated myocytes (16) have shown that action potentials in the crista terminalis have longer durations than those in the right atrial appendage (RAA) or free wall (pectinate muscle). Voltage-sensitive dye studies of the atria of the isolated sheep heart also identified left-to-right atrial differences in the frequency of activation of the two atria during atrial fibrillation (27). A subsequent study (37) demonstrated that this reflects left-to-right differences in the underlying cellular electrophysiology of myocytes from the left and right atrium.

Techniques for genetically altering the murine cardiovascular system have evolved substantially over the past decade (9, 30). As a result, murine models of cardiovascular physiology and pathophysiology, based on tissue-specific genetic manipulations, have become relatively common. This success has increased the need for semiquantitative methods for assessing the function of the murine cardiovascular system (20, 30). A recent study focusing on the ACh-activated K+ current (IK Ach) has demonstrated that atrial fibrillation in adult mice can be induced after administration of the IK Ach (muscarinic receptor) agonist carbachol (CCh) in wild-type animals, but not in IK Ach-deficient knockout mice (22). This finding suggests that shortening of atrial APD and/or hyperpolarization due to activation of IK Ach promotes atrial fibrillation in the mouse heart, as is the case in larger species, including humans (11, 27, 41). However, very little data is available concerning heterogeneity of APDs in murine atria compared with the amount of information available for the atria of larger species. Recent publications from our laboratory (10, 25, 26) based on whole cell patch-clamp recordings of K+ currents in isolated myocytes from the left and right atria of adult mice have provided some useful information. In these studies, left-to-right atrial gradients in several K+ currents, as well as differences in the APD of isolated myocytes, were reported. In particular, the inwardly rectifying K+ current and the rapidly activating sustained outward K+ current (IK W) were larger in myocytes from the left than from the right atrium. Partly for this reason, at a fixed rate of stimulation, action potentials in myocytes from the left atrium have shorter durations than those from the right atrium (25). In addition, myocytes from the right atrium had a significantly higher maximal density of the G protein-gated K+ current (IKG) than those from the left atrium (26).

We previously utilized voltage-sensitive dye imaging methods to study patterns of conduction in isolated mouse (31) and rat (33) hearts. In the present work, this voltage-sensitive dye imaging technique was adapted to monitor conduction and APDs in an isolated mouse atrial preparation (8, 25, 40). Our main objectives were as follows: 1) to determine whether the left-to-right differences in APD that we previously identified in isolated mouse atrial cells (25, 26) can also be demonstrated in...
intact mouse atrial tissue and 2) to identify and characterize intra- and interatrial spatial gradients in APD, which could not be resolved using the whole cell patch-clamp method. When single cells are the experimental preparation, only the atrium of origin (left or right), not the exact site of origin within each atrium, is known for each isolated myocyte. Our results provide important new information about intra-atrial gradients in APD in the adult mouse and confirm the presence of a left-to-right atrial gradient in the amplitude of $I_{K\text{ACH}}$. These findings provide new insights into our previously published observations of heterogeneity in mouse atrial APDs and are essential for relating electrophysiological findings in mouse atria to those obtained in larger mammals under physiological and pathophysiological conditions.

MATERIALS AND METHODS

Experimental preparation. Atrial preparations were isolated from 8- to 10-wk-old male C57BLK6 mice using methods that comply with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, Revised 1996) and with University of Calgary guidelines. Mice were anesthetized using methoxyflurane (Metavet) inhalation anesthesia and killed by cervical dislocation. The heart was immediately removed and placed in ice-cold Krebs solution of the following composition: 118.0 mM NaCl, 4.7 mM KCl, 1.2 mM KH$_2$PO$_4$, 1.2 mM MgSO$_4$, 1.0 mM CaCl$_2$, 25.0 mM NaHCO$_3$, 11.1 mM glucose, and heparin (10 U/ml). The lungs, thymus, and ventricular tissue were dissected away, leaving a preparation consisting of the interconnected left and right atria. This tissue was then pinned to the bottom of a Sylgard-coated 35-mm petri dish. Electrodes (Ag-AgCl loops) were attached to each atrium for electrogram recordings, and a stainless steel unipolar pacing electrode was positioned near the inferior vena cava. A typical preparation, including the electrode arrangement, is shown in Fig. 1A.

Superfusion with warm Krebs solution of the same composition described above, except without heparin, was then started at a constant flow rate of 8 ml/min, which was sufficient to maintain a bath temperature of 35 ± 1°C. The solution was continuously bubbled with 95% O$_2$-5% CO$_2$ in a 37°C water bath, resulting in pH 7.4. The Krebs solution passed through a glass heating coil (Radnoti Glass Technology, Monrovia, CA) immediately before entering the superfusion bath. The heating coil was warmed using a thermostimulator (Harvard Apparatus, Saint Laurent, PQ, Canada). The temperature in the bath near the preparation was monitored using a thermocouple thermometer (Harvard Apparatus) and recorded throughout the experiment. With this setup, the bath temperature could be controlled to within 1°C of the desired recording temperature (35°C). Soon after superfusion was begun, 200 nM ryanodine (Ryanodine Mixture, Calbiochem, San Diego, CA) was added to the superfusate. Over a period of ~20 min, this resulted in a substantial reduction in contraction amplitude [because of depletion of intracellular Ca$^{2+}$ stores (14, 19, 36)]. This maneuver suppressed motion artifacts sufficiently that APDs could be recorded and compared. During this period, the dye 4-[6-[2-(di-n-butylamino)-6-naphthylvinyl]pyridinium] (di-4-ANEPPS; Molecular Probes, Portland, OR) was also added to the superfusate for 10 min. Di-4-ANEPPS was prepared as a 10 mM stock solution in DMSO (stored frozen) and added to the standard Krebs solution (0.1% DMSO in the final solution).

Imaging system. The imaging system has been described in detail elsewhere (33). Briefly, illuminating light, provided by a 250-W quartz tungsten halogen light source (Oriel Instruments, Stratford, CT), is reflected off a cold mirror and filtered with a 500 ± 25 nm interference band-pass filter (Omega Optical, Brattleboro, VT). A dichroic mirror (Omega Optical) directs the band-pass-filtered light onto the preparation. A shutter (Oriel Instruments) is used to ensure that the preparation is exposed to light only during image acquisition.

The fluorescent light emitted from the preparation passes through the dichroic mirror and is further long-pass (>590 nm) filtered using a Schott glass filter (Melles Griot, Ottawa, ON, Canada) before reaching the camera.

A charge-coupled device camera (model CA-DI-0128T, Dalsa, Waterloo, ON, Canada) was used in its binning mode (60 × 60 pixels, 12-bit resolution, 950 frames/s). In the present application, it was equipped with a 25-mm focal length lens (Computar, Commack, NY) and a 5-mm spacer, which results in a 10 × 10 mm field of view (167 × 167 μm/pixel). Custom image acquisition software was written in LabVIEW (National Instruments, Austin, TX), and the camera was connected via an image acquisition board (model PCI-1422, National Instruments) to a personal computer (Pentium IV, 2 GHz, 1 GB RAM; Memory Express, Calgary, AB, Canada) running the Windows XP operating system (Microsoft, Redmond, WA). Electo-
trogram data, bath temperature, and the trigger signal for the stimulator were recorded using a real-time data acquisition board (model PCI-7030/6030E, National Instruments) synchronized with the camera.

**Data processing.** Data were processed offline using software written in the IDL development environment (Research Systems, Boulder, CO), with external routines written in C++ (Visual C++, Microsoft) for the most computationally demanding tasks. The data recorded for each individual pixel were processed as follows: 1) subtraction of background fluorescence, 2) linear trend removal (trend estimated by least-squares fitting a straight line to the data), and 3) sign change, so that a depolarization corresponded to a positive change in the signal. The linear trend removal step was included as a safeguard against baseline changes due to photobleaching. However, with the low light intensity used in this study, no significant photobleaching was observed, and this processing step was not important to the results presented here. Because the preparation did not fully utilize the available field of view, areas not covering the preparation were manually masked and excluded from further analysis. Signal-averaged data were computed from all recordings by averaging all cycles acquired during a recording period of 10 s, with the stimulus pulse (paced rhythms) or the electrogram (sinus rhythm) used as a time reference. The activation time for each pixel was then detected from the signal-averaged data as the time of maximum rate of rise of the fluorescence signal (31). Isochrones were computed from the signal-averaged maps with use of a built-in contour plot routine in IDL.

Local conduction velocity vectors were computed from the slope of a plane least-squares fit to $7 \times 7$ pixel areas of the activation maps as described previously (29, 31). To compare the conduction velocity in a preparation in sinus rhythm under different conditions, the average magnitude of the local conduction velocities within each atrium was computed without accounting for the direction (assuming that this predominantly represents conduction along fiber directions).

Repolarization times were detected as the time of crossing a level corresponding to 70% repolarization. The detection was based on a low-pass-filtered (5-point moving-average smoothing) version of the signal-averaged data to avoid spurious detections due to remaining noise. Action potentials at individual pixel locations affected by remaining motion artifact or unusually low signal-to-noise ratio were excluded on the basis of the following heuristic criteria applied to the signal-averaged data as the time of maximum rate of rise of the fluorescence signal (31). Isochrones were computed from the signal-averaged maps with use of a built-in contour plot routine in IDL. Local conduction velocity vectors were computed from the slope of a plane least-squares fit to $7 \times 7$ pixel areas of the activation maps as described previously (29, 31). To compare the conduction velocity in a preparation in sinus rhythm under different conditions, the average magnitude of the local conduction velocities within each atrium was computed without accounting for the direction (assuming that this predominantly represents conduction along fiber directions).

Repolarization times were detected as the time of crossing a level corresponding to 70% repolarization. The detection was based on a low-pass-filtered (5-point moving-average smoothing) version of the signal-averaged data to avoid spurious detections due to remaining noise. Action potentials at individual pixel locations affected by remaining motion artifact or unusually low signal-to-noise ratio were excluded on the basis of the following heuristic criteria applied to the signal-averaged, but unfiltered, signal: 1) the signal must remain at $<30\%$ of maximum amplitude until 15 ms before the detected activation (maximum rate of rise) time (to exclude signals with unstable baseline and/or large noise), 2) the signal must reach $>90\%$ of maximum amplitude within 10 ms after the activation time (to exclude signals with very slow upstrokes), and 3) the signal must return (and remain) to $<30\%$ of maximum amplitude by 80 ms after the activation time (to exclude signals with unstable baseline, large noise, or unreasonably long apparent APDs due to motion artifacts). APDs were then computed as the intervals between the activation time and the repolarization time detected for each pixel.

The analysis software allows the user to select regions corresponding to anatomic features and computes summary statistics for the APDs within each selected region. Four regions were used to facilitate statistical comparisons within and between preparations throughout the study: 1) the RAA, the right atrial tissue anterior to the sulcus terminalis [this area contains the trabeculated part of the right atrial wall referred to as the “right atrial free wall” in some publications (16, 18)]; 2) the right atrial posterior wall (RAPW), the right atrial tissue bordered on the right by the sulcus terminalis and on the left by the groove between the superior vena cava and the right pulmonary veins; 3) the left atrial posterior wall (LAPW), the left atrial tissue bordered on the right by the groove between the superior vena cava and the right pulmonary veins, extending anteriorly up to the narrow junction with the left atrial appendage (LAA) to include the left pulmonary veins; and 4) the LAA, the trabeculated left atrial tissue anterior to the narrow junction separating the appendage from the smooth-walled part of the left atrium receiving the pulmonary veins. Because the division into these four regions was done on the basis of the relatively low-resolution optical mapping data, the structures delineating the regions could only be identified approximately on the basis of the outline of the preparation.

Values are expressed as means ± SE. Hypothesis testing was carried out using unpaired or paired t-tests as appropriate (in cases with only 2 groups) and ANOVA or repeated-measures ANOVA followed by Holm’s t-test for three or more groups. $P < 0.05$ was considered to be statistically significant.

**RESULTS**

**Long-term stability of APDs.** Control experiments were done to evaluate the long-term stability of APDs in this in vitro atrial preparation. Six recordings, at a fixed pacing interval of 130 ms, were made at 10-min intervals in two preparations that were not subjected to drug application other than ryanodine and CCh, where each drug was applied for 10 min before recording. Therefore, the influence of any long-term changes in APDs in interpreting our results is considered to be negligible. The cycle length in sinus rhythm was also evaluated in three control preparations under the conditions described above as a further measure of the viability of the preparation. Sinus rate in these three preparations slowed gradually from a cycle length of 205 ± 20 (SE) ms at the beginning of the experiment to 234 ± 14 ms after 50 min.

**Activation patterns in sinus rhythm.** In the first series of experiments, atrial activation patterns were recorded during sinus rhythm to characterize the physiological sequence of activation and determine conduction velocities in this isolated mouse atrial preparation. Figure 2 summarizes these results. Activation originated in a site on the right atrium near the sulcus terminalis, marked by the constriction at the border between the trabeculated RAA and the smooth posterior wall between the caval veins (Fig. 2A). The exact location of this site of initial activation varied slightly between preparations, sometimes being closer to the inferior vena cava (Fig. 2A) and sometimes closer to the superior vena cava (cf. Fig. 3A). However, activation always originated from a site near the sulcus terminalis in the right atrium, as expected from the known anatomic location of the sinoatrial (SA) node in the adult mouse heart (40). The impulse then spread approximately radially throughout the wall of the RAA. After a brief delay, the impulse propagated across to the left atrium and then spread radially throughout the wall of the LAA. This pattern of activation was very consistent between preparations ($n > 20$).
However, the conduction delay from the right to the left atrium was somewhat more variable. It is possible, therefore, that the right-to-left delay in our preparations was affected by the dissection procedure and/or by the slight stretch caused when the preparation was secured (pinned) to the bottom of the superfusion chamber. In Fig. 2C, the isochronal contour map from Fig. 2A is superimposed on an image of the preparation. This provides a reference to important anatomic landmarks within this type of preparation.

To verify that conduction in the isolated atrial preparations exhibited normal physiological behavior and to ensure that our superfusion system could change solutions effectively within a few minutes, we also carried out these same measurements using a Krebs solution with extracellular K⁺ concentration ([K⁺]₀) increased to 12 mM by addition of KCl. The overall pattern of activation was very similar under these conditions (Fig. 2B). However, as expected, the conduction velocity within each atrium was slowed, and the delay from the right to the left atrium was increased by the elevated [K⁺]₀. Figure 2D shows summary data for conduction velocity measured in the atrial appendages as described in MATERIALS AND METHODS. An increase in [K⁺]₀ from the normal 5.9 mM to 12 mM resulted in a statistically significant slowing of conduction velocity in both atrial appendages. Conduction velocities in normal (5.9 mM) [K⁺]₀ were 0.44 ± 0.08 and 0.41 ± 0.04 mm/ms in the RAA and LAA, respectively, and slowed to 0.25 ± 0.07 and 0.27 ± 0.01 mm/ms, respectively, in 12 mM extracellular K⁺. No significant differences in conduction velocity between the RAA and the LAA were observed.

Heterogeneity of atrial APDs. The use of ryanodine (200 nM) to suppress motion artifacts, in combination with signal averaging (see MATERIALS AND METHODS), resulted in an action potential signal of sufficient quality to allow accurate measurement of the APD in most pixels. Figure 3C shows a sample set of action potentials across a representative preparation. These signals were of good quality, except near the edges of the preparations. However, in some preparations, the signal-to-noise ratio was low in parts of the posterior wall area. This is most likely due to difficulties in dissecating away fatty tissue that covered this area in these hearts. We did not observe any trend for the signal quality in this area to deteriorate with time during the experiment and, therefore, consider it unlikely that the lower signal quality was due to inadequate superfusion. Figure 3C also illustrates the criteria used to exclude action potentials affected by residual motion artifact or low signal-to-noise ratio (see MATERIALS AND METHODS).

APDs were quantified throughout the isolated atrial preparations as described in MATERIALS AND METHODS, and maps of APDs were constructed. An example is shown in Fig. 3, which demonstrates a general and consistent trend: the APDs are shorter in the two appendages than in the posterior walls. To facilitate comparisons across multiple preparations, each preparation was subdivided into four regions (Fig. 3). The average APD within each region was computed for each preparation, and this parameter was used for statistical comparisons. Figure 3D shows summary data of the APDs in the 4 regions in 21 preparations. These results demonstrate significant heterogeneity in the APDs within each atrium and between the left and the right atrium. A consistent finding is longer APDs in the posterior wall regions (LAPW and RAPW) than in the atrial appendages (LAA and RAA). Furthermore, there appears to be a right-to-left gradient in the posterior wall region, such that APDs are longer in the RAPW than in the LAPW. Comparison of the APDs in the atrial appendages also reveals a small (~1 ms), but statistically significant, right-to-left difference, with the RAA exhibiting longer APDs at this stimulus interval (130 ms) and temperature (35°C).
Response to $K^+$ channel blockers. After having established that this experimental approach yielded consistent data concerning activation, conduction, and APD, the effects of the $K^+$ channel blockers 4-aminopyridine (4-AP) and tetraethylammonium (TEA) on the APDs in the isolated mouse atrial preparations were examined (Fig. 4). Both of these compounds were selected because they are known to block repolarizing $K^+$ currents in mouse atrial myocytes (6, 25). 4-AP (100 μM) caused a statistically significant prolongation of APDs (by 2 ms) in all four regions considered in this study at a stimulus interval of 130 ms. No statistically significant regional heterogeneity in the ability of this concentration of 4-AP to prolong the APD was observed between the selected atrial regions ($n = 9$). TEA (5 mM) caused statistically significant APD prolongation of 5–6 ms in the appendages and 8–9 ms in the posterior wall regions ($n = 3–5$). However, as in the case of 4-AP, no statistically significant regional heterogeneity in the response to TEA was detected, although there is a possible trend for the TEA response to be larger in the posterior wall areas.

Heterogeneity of response to muscarinic cholinergic stimulation. Our previous study of isolated cells from mouse left and right atria identified differences in the maximal responses to CCh (10 μM) between the left and the right atrium. On the basis of these observations, we determined the effect of a maximal dose (10 μM) of CCh on APDs in isolated atrial preparations. In these experiments, addition of CCh to the superfusate was followed (after a maximal CCh effect had developed) by application of a muscarinic receptor antagonist. Three different antagonists were used: methoctramine (1 μM), atropine (1 μM), and scopolamine (1 μM). ANOVA comparing the results obtained with these three different antagonists did not reveal any statistically significant differences. Therefore, data collected using all three antagonists were pooled. Figure 5, A and B, shows typical action potentials recorded from the RAA and LAA, respectively. The action potential in
the action potential of the LAA changed very little. Figure 5A shows the RAA shortened substantially in response to CCh, whereas Fig. 4. Effect of K⁺ channel blockers on APD in mouse atria. A: prolongation of APD by 100 μM 4-aminopyridine (4-AP, n = 9), B: prolongation of APD by 5 mM tetraethylammonium (TEA, n = 3–5). *Significantly different from control for each region, P ≤ 0.05.

the RAA shortened substantially in response to CCh, whereas the action potential of the LAA changed very little. Figure 5C consists of a summary of these data. CCh caused a statistically significant shortening of APD in all regions except the LAA. In this region, only a very small shortening was observed, and this CCh-induced change failed to reach statistical significance. A small shortening of the APD in the LAA was observed in some individual preparations, but this response was always considerably smaller than that observed in the RAA in the same preparation. After application of a muscarinic receptor antagonist, the APDs lengthened in all regions, including the LAA. APDs in the presence of the muscarinic antagonist exceeded the control values (statistically significant) in all regions (Fig. 5C). The average “overshoots” (APD after CCh and muscarinic antagonist — control APD) for the four regions were as follows: 3.38 ± 0.50, 4.42 ± 0.71, 3.22 ± 0.70, and 4.39 ± 0.76 ms for the RAA, RAPW, LAPW, and LAA, respectively. There was no significant regional difference in the size of this response (P = 0.14). In two preparations (data not shown), a muscarinic receptor antagonist was applied without prior application of CCh. The APDs (average across the entire preparation) in these experiments remained within ±1 ms, indicating no significant contribution from endogenous ACh release under our control conditions. As outlined in DISCUSSION, this is a well-known response that has been observed in several different atrial preparations, although the underlying mechanisms remain a subject of some controversy.

DISCUSSION

Baseline characteristics of the mouse atrial preparation. Control experiments were done to evaluate the long-term stability of APD under these experimental conditions, because measurement of this parameter was essential in this study. The results show that average APD₇₀ (at 35°C and 130-ms stimulation interval) remains stable (within 1 ms) at ~14 ms over a 50-min period, i.e., considerably longer than the time required to complete an individual experiment. The recordings used to quantify APDs were carried out during paced rhythm with a fixed stimulus interval (130 ms), thus eliminating any contributions to the results from changes in sinus rate and possible rate dependence of the APD. To provide additional evidence that these in vitro atrial preparations exhibited normal electrophysiological behavior, we have also recorded activation patterns during sinus rhythm. These recordings (Fig. 2A) show that activation (defined by breakthrough of activation onto the epicardial surface visible to the camera) originates at a site in the right atrium near the crista terminalis. The activation wavefront then spreads radially throughout the RAA and, after a delay, moves into and across the LAA. This is consistent with the known sequence of atrial activation in, e.g., the canine heart, in which the impulse originates in the SA node, spreads into the right atrium via the crista terminalis, and crosses over to the left atrium via one of the known interatrial pathways (2, 13, 39). Our results also appear to agree with a recent detailed description of electrophysiological activation of the adult mouse SA node and right atrium (40).

Our experimental preparation is superfused, as opposed to arterially perfused. It was important, therefore, to demonstrate its response to solution switching (e.g., drug application) and determine the time course of this maneuver before beginning pharmacological measurements. To address this issue, experiments were done in which the K⁺ concentration in the superfusing solution was increased to 12 mM from the baseline of 5.9 mM. The activation pattern in sinus rhythm remained approximately the same under these conditions, and, as expected, the conduction velocity slowed significantly (from 0.44 ± 0.08 to 0.25 ± 0.07 mm/ms in the right atrium and from 0.41 ± 0.04 to 0.27 ± 0.01 mm/ms in the left atrium). This very-well-known response, due to depolarization of the resting membrane potential (32) and subsequent reduction in the availability of inward Na⁺ current, confirms that, under our experimental conditions, an effective change of solution throughout the preparation is complete within a few minutes. This finding and the demonstration that APD₇₀ remains very stable over 50 min provide strong evidence that this isolated atrial preparation is adequately superfused.

There are two previous reports of atrial conduction velocities obtained in in vitro preparations of the SA node and right atrium (40) or the Langendorff-perfused adult mouse heart (1). The first of these studies (40) reports values of 0.50–0.65 mm/ms within the SA node and 0.80–0.90 mm/ms in the periphery of the SA node, where atrial cells were identified (at 37°C). These measurements were made in a small endocardial
region in the immediate vicinity of the SA node and cannot be
directly compared with our results. Alcolea et al. (1) report a
conduction velocity of $0.31 \pm 0.02$ mm/ms in the right atrium
and $0.32 \pm 0.02$ mm/ms in the left atrium. Our values, $0.44 \pm
0.08$ mm/ms for the right atrium and $0.41 \pm 0.04$ mm/ms (Fig.
2D) for the left atrium, are comparable to their results.

We have used a low (200 nM) concentration of ryanodine to
reduce contraction amplitude and, thus, limit motion artifacts
in our recordings. We acknowledge that this maneuver alters
the intracellular Ca$^{2+}$ transient and may, as a result, shorten the
APD by reducing the inward current contributed by the Na$^+$/
Ca$^{2+}$ exchanger during repolarization. We believe that this
effect is small in a rapidly repolarizing (“triangular”) action
potential, such as that observed in the mouse atrium. The
reason is that the outward currents underlying the very rapid
repolarization of this type of action potential are very large
compared with the inward current contributed by the Na$^+$/
Ca$^{2+}$ exchanger. Thus, although there is a possibility that the
use of ryanodine resulted in a minor shortening of the APD in
our recordings, this effect is small compared with the substan-
tial lengthening resulting from the alternatives (2,3-butanedi-
one monoxime or cytochalasin D) (3).

**Heterogeneity of APDs in mouse atria.** Our measurements of
APDs at a number of sites within the isolated atrial prepara-
tions yield several consistent new findings: 1) The APD$_{70}$ is
shorter in the two atrial appendages than in the posterior wall
regions. 2) The RAPW region has an average APD$_{70}$ that is
longer than that of the corresponding area of the left atrium. 3)
There is evidence of a small (~1 ms), but statistically signif-
ificant, difference in APD$_{70}$ between the atria, with the RAA

---

**Fig. 5.** Heterogeneity of changes in APD in response to car-
bachol (CCh). A: action potentials from 1 selected pixel in the
RAA, in which 10 $\mu$M CCh causes a significant shortening of
APD$_{70}$. This effect was reversed by 1 $\mu$M methoctramine
(Meth). B: action potentials from 1 selected pixel in the LAA,
in which the response to the same concentration of CCh is much
smaller than in the RAA. C: summary data ($n = 11$–12 for
RAA and LAA, $n = 7$–8 for RAPW and LAPW) showing
APD$_{70}$ response to 10 $\mu$M CCh, followed by 1 of the 3
muscarinic antagonists (methoctramine, scopolamine, or atro-
pine). *Significantly different from control, $P \leq 0.05$. **Signif-
ically different from CCh, $P \leq 0.05$. 

---

**AJP-Heart Circ Physiol • VOL 287 • DECEMBER 2004 • www.ajpheart.org**
having a slightly longer APD$_{70}$ than the LAA. Longer APDs and/or effective refractory periods in the right than in the left atrium has been observed consistently across a wide range of species (Table 1). Our observations in the present multicellular preparation, as well as in isolated mouse atrial myocytes (25), confirm that this left-to-right difference exists in the mouse atria as well.

**Gradients within the right atrium.** Within the right atrium, APD$_{70}$ was shorter in the appendage than in the posterior wall area or the area overlying the crista terminalis. This finding is consistent with published observations from the atria of larger mammals. Hogan and Davis (18) reported a shorter APD for the canine right atrial free wall (pectinate muscle contained within the RAA region in our study and the atrial roof area) than at the caval border of the crista terminalis. Spach et al. (38) made similar observations. These authors describe an overall trend for APDs to decrease with increasing distance from the SA node in the canine right atrium. More recently, Feng et al. (16) also found that canine crista terminalis exhibits longer APDs than the free wall of the RAA. Using the whole cell patch-clamp technique, these authors also investigated the ionic mechanisms underlying this difference. They reported 1) a greater density of the inward L-type Ca$^{2+}$ current in the crista terminalis than in the free wall of the appendage and 2) a greater density of the outward K$^+$ current in the free wall. In the rabbit right atrium, Yamashita et al. (45) observed the same trend: APDs are longer in the crista terminalis than in the RAA. In the rabbit, voltage-clamp measurements showed that the underlying cause for this gradient was a corresponding gradient in the amplitude of the transient outward current. Our results obtained in the mouse right atrium are thus consistent with existing literature for canine and rabbit right atrium. The ionic mechanism, however, remains to be identified in the adult mouse heart.

**Gradients within the left atrium.** Results based on optical recordings within the left atrium show a tendency similar to that in the right atrium; i.e., APD$_{70}$ is considerably shorter in the LAA than in the LAPW. There is little information in the literature concerning heterogeneity of APDs within the left atrium. A related topic that has received considerable attention recently is the possible role of electrophysiological activity in the pulmonary veins in triggering and/or sustaining atrial fibrillation (17). Ehrliech et al. (15) compared action potentials from myocytes obtained in the left atrial free wall with those from myocytes obtained from the pulmonary vein “sleeve.” Their measurements indicate that APDs of cardiac myocytes from this pulmonary vein tissue are shorter than those from the left atrial free wall. This finding, if it applies to the mouse heart, appears to contradict our observation that the LAA has a shorter APD$_{70}$ than the LAPW (which encompasses the pulmonary veins). However, in our analysis, the pulmonary veins account for a small fraction of the total area included in the posterior wall region. If APDs are longer in other parts of the LAPW region, this would likely prevent the detection of shorter action potentials in the pulmonary vein sleeves with our spatial resolution. However, it is possible that shorter APDs in the pulmonary vein sleeves may have contributed to our observation that average APDs are shorter in the LAPW than in the RAPW.

**Response to K$^+$ channel blockers.** Prolongation of the action potentials within the isolated atrial preparations in response to the K$^+$ channel blockers 4-AP and TEA was measured in detail. On the basis of data from mouse atrial myocytes (6, 25), these channel blockers would be expected to cause substantial prolongation of the APD. 4-AP is known to block $I_{K,ur}$, carried by K$^+$ channels, the α-subunit of which is most likely encoded by K$_{ur}$ (6, 25). TEA has been shown to block the 4-AP-insensitive sustained K$^+$ current in mouse atrium, likely carried by K$^+$ channels encoded by K$_{ur}$ (21). TEA (5 mM) caused a statistically significant prolongation of APD$_{70}$ of 5–6 ms in the atrial appendages and 8–9 ms in the posterior wall regions. Although the average prolongation of the APD was somewhat longer in the posterior wall regions than in the appendages, this difference did not reach statistical significance. 4-AP (100 μM) caused a statistically significant, but relatively small (~2 ms), prolongation of APD$_{70}$ in all regions of the atrial preparation. No regional heterogeneity in this response was observed.

The small response to 100 μM 4-AP in the present study differs from that observed in our previous work in isolated mouse atrial myocytes (25). In the isolated atrial myocytes, this concentration of 4-AP caused a ~10- to 15-ms prolongation of APD at 90% repolarization, i.e., a much more substantial prolongation of the APD. Furthermore, this response exhibited regional heterogeneity, such that the response was almost twofold larger in myocytes isolated from the left atrium than in those isolated from the right atrium. Given that the response to 4-AP in this study is much smaller, it is to be expected that we are unable to identify regional heterogeneity in the response to 4-AP. The reasons underlying this difference in the size of the

### Table 1. Summary of left-to-right differences in atrial ERP and APD reported previously in the literature

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Ref.</th>
<th>RA, ms</th>
<th>LA, ms</th>
<th>$\Delta$, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open-chest dogs, multiple recording sites</td>
<td>23</td>
<td>120–130</td>
<td>114–116</td>
<td>−10</td>
</tr>
<tr>
<td>CL = 200 ms</td>
<td>134–143</td>
<td>123–129</td>
<td>$-12$</td>
<td></td>
</tr>
<tr>
<td>CL = 250 ms</td>
<td>140–150</td>
<td>139–143</td>
<td>$-4$</td>
<td></td>
</tr>
<tr>
<td>Langendorff-perfused rabbit hearts (37°C)</td>
<td>35</td>
<td>82.2±9.8</td>
<td>69.7±5.8</td>
<td>−12</td>
</tr>
<tr>
<td>CL = 250 ms</td>
<td>200 ms</td>
<td>16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL = 250 ms</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL = 300 ms</td>
<td>22</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL = 400 ms</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Open-chest pigs</td>
<td>44</td>
<td>35±2</td>
<td>39±2</td>
<td>38±2</td>
</tr>
<tr>
<td>Single canine atrial myocytes (35°C)</td>
<td>24</td>
<td>170.9±4.3</td>
<td>152.9±4.3</td>
<td>−18</td>
</tr>
<tr>
<td>CL = 1 s</td>
<td>173.3±8.7</td>
<td>140.0±8.7</td>
<td>−33</td>
<td></td>
</tr>
<tr>
<td>Multicellular canine atrial preparations (37°C)</td>
<td>24</td>
<td>173.3±8.7</td>
<td>140.0±8.7</td>
<td>−33</td>
</tr>
<tr>
<td>CL = 1 s</td>
<td>173.3±8.7</td>
<td>140.0±8.7</td>
<td>−33</td>
<td></td>
</tr>
<tr>
<td>Single mouse atrial myocytes (22°C)</td>
<td>25</td>
<td>19.8±1</td>
<td>16.9±0.9</td>
<td>−3</td>
</tr>
</tbody>
</table>

ERP, effective refractory period; APD, action potential duration; APD$_{90}$, APD at 90% repolarization; RA, right atrium; LA, left atrium; $\Delta$, difference between right and left atrium; CL, cycle length.

**AJP-Heart Circ Physiol • VOL 287 • DECEMBER 2004 • www.ajpheart.org**
4-AP response between the present results and those obtained in isolated myocytes are not clear. The observation that 5 mM TEA is able to produce a substantial increase in APD_H2, as well as the observation that the preparations respond as expected to an increase in [K^+]_0, in the superfuse, provides evidence that our superfusion system is capable of changing the bath composition effectively. It therefore seems highly unlikely that the small response to 4-AP is due to difficulties in delivering the channel blocker to the preparation. Another possibility that must be considered is that the amplitude of the 4-AP-sensitive K^+ current (I_{K,4AP}) was suppressed in our preparations already under control conditions. This appears very unlikely as, if we consider that the control APD values in the present study are comparable to, or even shorter than, the control APDs measured in isolated myocytes (25). One difference between the recording conditions used in the present study and those used in the experiments on single myocytes (25) is that the present work was carried out at near-physiological temperature (35°C), whereas the studies on single myocytes were carried out at room temperature. This difference in temperature may have affected the kinetics of the drug-channel interaction, and also required the use of different stimulation rates in the two studies. It is possible that these differences in recording conditions are the cause of the difference in the response to 4-AP between the two preparations.

**Heterogeneity of response to CCh.** A maximal dose of CCh (10 μM) caused a statistically significant shortening of the APD in all regions of the mouse atrium that were examined in this study, except the LAA (Fig. 5). Subsequent application of a muscarinic antagonist resulted in lengthening of the APDs to values greater than the control values in all parts of the preparation. This overshoot phenomenon has been identified previously in a number of different preparations (5, 42). The mechanism underlying this phenomenon remains a subject of some debate (4, 5, 12, 42) and, in fact, may differ between species and/or between atrial and ventricular tissue in the mammalian heart.

The heterogeneity in the response to CCh observed in the present study is consistent with our previous observations in isolated mouse atrial myocytes (26), in which 10 μM CCh evoked a significantly larger I_{K,ACH} in myocytes isolated from the right atrium than in those isolated from the left atrium. The present results shed additional light on this observation by demonstrating that there is a gradient within the left atrium, such that there is a significant response to CCh in the posterior wall area of the left atrium, but not in the appendage. However, this observation is the opposite of the results reported in sheep atria by Sarmast et al. (37), who reported larger I_{K,ACH} in myocytes isolated from the left than from the right atrium. The significance of this species difference is not clear. Heterogeneity in the magnitude of I_{K,ACH} in general may serve to create increased dispersion of repolarization in response to vagal stimulation and, thus, create a substrate for reentrant arrhythmias (7). The observation that atrial fibrillation can be induced in the presence of CCh in wild-type, but not in I_{K,ACH}-deficient knockout, mice (22) demonstrates that I_{K,ACH} has an important role in inducibility of atrial arrhythmias. A recent modeling study (21) has also demonstrated that I_{K,ACH}-induced heterogeneity in atrial refractoriness can have a role in maintaining atrial fibrillation.

**Summary.** Our results validate a new approach for monitoring activation and repolarization in an isolated mouse atrial preparation based on voltage-sensitive dye mapping. The activation patterns are consistent with those observed in larger mammals, as well as with previous observations in an isolated mouse SA node-right atrium preparation (40). We have also observed a heterogeneous pattern of APDs that is consistent with observations in canine (18, 38) and rabbit (45) atria. Finally, the response to CCh in the LAA is very small compared with that in the RAA, confirming our previous observations in isolated mouse atrial myocytes (26). These results demonstrate that the electrophysiological and pharmacological properties of mouse atria are quite similar to those of larger animals. Further studies using this preparation and recording technique are therefore warranted.

**ACKNOWLEDGMENTS**

We thank Colleen Kando for excellent technical assistance.

**GRANTS**

This work was supported by grants from the Canadian Institutes for Health Research, the Heart and Stroke Foundation of Canada (HSFC), and the Alberta Heritage Foundation for Medical Research (AHFMR). A. E. Lomax held a fellowship from the AHFMR, and A. Nygren was a HSFC Fellow.

**REFERENCES**


