Cellular electrophysiologic properties of old canine atria provide a substrate for arrhythmogenesis

Evgeny P. Anyukhovsky\textsuperscript{a}, Eugene A. Sosunov\textsuperscript{a}, Alexei Plotnikov\textsuperscript{a}, Ravil Z. Gainullin\textsuperscript{a}, Jeffrey S. Jhang\textsuperscript{b}, Charles C. Marboe\textsuperscript{b}, Michael R. Rosen\textsuperscript{a,\,*}

\textsuperscript{a}Department of Pharmacology, Center for Molecular Therapeutics, College of Physicians and Surgeons of Columbia University, 630 West 168th Street, PH 7 West-321, New York, NY 10032, USA

\textsuperscript{b}Department of Pathology, College of Physicians and Surgeons of Columbia University, New York, NY 10032, USA

Received 31 October 2001; accepted 10 January 2002

Abstract

Objective: The incidence of atrial fibrillation increases with age. We hypothesized that aging-associated changes in the atrial action potential (AP) and conduction velocity provide a substrate for abnormal conduction and arrhythmogenesis. Methods: We used microelectrode techniques to record AP from the endocardium of the right atrial wall of dogs aged 1–5 (adult) and >8 years (old). Conduction velocity was measured between two microelectrodes 3–10 mm apart. Histological study was carried out to assess fibrosis. Results: Whereas resting potential, AP amplitude and $V_{max}$ did not differ with age, the plateau was more negative and AP duration was longer in old tissue. The L-type calcium current ($I_{Ca,L}$) agonist Bay K8644 ($10^{-8}–10^{-6}$ mol/l) elevated the plateau and shortened APD more in old than in adult, such that AP contour in old atria approached that of adult. In contrast, the $I_{Ca,L}$ blocker nisoldipine ($10^{-8}–10^{-5}$ mol/l) depressed the plateau in adult and had no effect in old. There was no difference between the two groups in conduction velocity of normal beats, whereas for early premature impulses, reduced conduction velocity and a wider time window manifesting slow conduction were detected in old in comparison to adult tissue. A twofold increase in the amount of fibrous tissue was detected in old atria. Conclusions: Our data show significant differences in contour of AP in adult and old atria. The responses to Bay K8644 and nisoldipine suggest a decreased $I_{Ca,L}$ in old atrial tissue. The alterations in AP contour and increased fibrosis may be responsible for slower conduction of early premature beats in old atria. The age-related changes in conduction of premature beats are consistent with those observed in patients with paroxysmal atrial fibrillation and may contribute to the greater propensity to atrial fibrillation in the aged. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Arrhythmia (mechanisms); Atrial function; Membrane potential; Connective tissue; Aging

1. Introduction

Only a few studies of age-induced cardiac electrophysiological changes have been performed, and their results are somewhat inconsistent. For example, in senescent canine hearts, the Purkinje fiber action potential (AP) plateau is lower than that in adults [1], and atrial myocardial L-type Ca current is reduced [2]. In contrast, AP prolongation and an increase in calcium current have been observed in senescent rat ventricular myocardium [3–5].

No AP duration (APD) changes have been reported in senescent compared to adult rat atria [6,7].

The goal of the present study was to determine whether the AP characteristics of normal old atria provide a substrate conducive to atrial arrhythmias, particularly atrial fibrillation, which occurs most frequently in older individuals [8–11]. Its incidence is 0.2–0.3% at age 25–35 and increases monotonically to about 10% by age 80 [9,11]. The higher likelihood of atrial fibrillation in the elderly may reflect a variety of factors, including disease, fibrosis and/or age-related changes in cellular electrophysiologic properties, such that disturbances of atrial

*Corresponding author. Tel.: +1-212-305-8754; fax: +1-212-305-8351.
E-mail address: mrr1@columbia.edu (M.R. Rosen).

Time for primary review 22 days.

0008-6363/02/$ – see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S0008-6363(02)00271-7
activation and repolarization are likely sequellae. The dog was selected as the experimental model because it develops atrial fibrillation as a result of disease [12] and with aging [13,14], because its cellular electrophysiologic properties are akin to those of the human, and because canine models of atrial fibrillation are extensively used in studying the cellular and molecular changes associated with atrial fibrillation [15–18].

2. Methods

2.1. Experimental preparations

The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Two groups of mongrels of either sex weighing 16–22 kg were investigated: adult dogs (1–5 years) and old dogs (>8 years). The ages of the dogs were estimated based on physical examination. Six-lead ECG measurements were made on conscious dogs resting quietly on the right side using the Dr Vetter software (Dr Vetter, Baden-Baden, Germany). Rate correction of the QT interval was done using Bazett’s formula.

After ECG recordings, animals were anesthetized with sodium pentobarbital (30 mg/kg i.v.). Their hearts were removed through a left lateral thoracotomy and immersed in warm (35 °C) Tyrode’s solution equilibrated with 95% O₂/5% CO₂ and containing (mmol/l): NaCl 131, NaHCO₃ 18, KCl 4, CaCl₂ 2.7, MgCl₂ 0.5, NaH₂PO₄ 1.8 and dextrose 5.5. Tissue strips from pectinate muscles of the right atrial free wall (~10×5×0.5 mm) were then dissected. The preparations were placed in a tissue bath endocardial side up and superfused with control Tyrode’s solution (r=37 °C, pH 7.35±0.05). Solution was pumped at 12 ml/min, changing chamber content three times/min. The bath was connected to ground via a 3 M KCl/Ag/AgCl junction.

2.2. Action potential recordings

Preparations were impaled with 3 mol/l KCl-filled glass capillary microelectrodes that had tip resistances of 10–20 MΩ. The maximum upstroke velocity of the AP (V_max) was obtained by electronic differentiation with an operational amplifier. The electrodes were coupled by an Ag/AgCl junction to an amplifier with high input impedance and input capacity neutralization. Transmembrane action potentials and V_max signals were digitized with an analog-to-digital converter (D-210, DATAQ Instruments Inc) and stored to PC for subsequent analysis. For stimulation of preparations, standard techniques were used to deliver square-wave pulses 1.0 ms in duration and 1.5 times threshold through bipolar PTFE-coated silver electrodes.

To investigate frequency-dependence of drug effects, the preparations were driven at cycle lengths of 2000, 1000, 500 and 250 ms in sequence. Each frequency was maintained for 3 min before data were collected.

Experiments were started after 2 h of superfusion in control Tyrode’s solution when preparations had fully recovered and displayed stable electrophysiological characteristics. Preparations with maximum diastolic potential (MDP) positive to −75 mV at a cycle length of 1000 ms were not used for experiments. The cumulative effects of the L-type calcium current (I_{Ca,L}) agonist Bay K8644 [19] (10⁻⁸–10⁻⁶ mol/l) and antagonist nisoldipine [20] (10⁻⁸–10⁻⁶ mol/l) were studied. Measurements of drug effects commenced after the preparations had equilibrated for 30 min at each concentration.

2.3. Measurements of conduction velocity and membrane responsiveness

Strips of pectinate muscle 8–15 mm long and 1.5–2.0 mm wide were used for the experiments. Microelectrode impalements were made at two sites 3–10 mm apart along the longitudinal axis of the preparation. Preparations were stimulated at a basic cycle length of 1000 ms at a site >2 mm from the proximal microelectrode and premature stimuli (2 ms duration and three times threshold amplitude) were introduced progressively earlier during repolarization after a train of 10 regularly occurring stimuli. Conduction time of the basic drive impulse and of premature impulses was defined as the time between two V_max signals. Conduction velocity was calculated by dividing the interelectrode distance, measured with a reticle mounted in a microscope, by the conduction time. V_max and the takeoff potential of premature action potentials were measured to construct the membrane responsiveness curve. The earliest propagated premature response was assumed to demarcate the end of the effective refractory period (ERP).

2.4. Histology

Biopsy material from the right atrial free wall of adult and elderly dogs was formalin-fixed and paraffin embedded. Sections were cut at 5-μm thickness and stained with a Masson trichrome stain with myocytes staining red and collagen staining blue. In a pilot study of four cases, multiple representative 10× fields were chosen and digitally captured with an Olympus digital camera. Images were digitally manipulated to remove endocardium, which spuriously increases the amount of fibrosis calculated. The images were then compressed to 0.3 megapixel images in JPEG compression format. Software was then developed to analyze the digitally captured, JPEG compressed images. The software program digitally assigned the total number of pixels in the image to ‘red’ pixels corresponding to myocardium and ‘blue’ pixels corresponding to collagen.
White space was ignored and not counted by this program. The software presented visual feedback of the image and sensitivities for the detection of red pixels (myocardium) and blue pixels (connective tissue) were adjusted during the analysis for optimal assignment of red and blue pixels. The amount of fibrosis present was calculated as a percentage of the absolute number of pixels assigned as red to the absolute number of pixels assigned as blue. The pilot study of four cases was studied with a range of 4–6 10×, randomly selected images assessed per case. The average S.D. of the random fields for all four cases were 1, 1, 1.9 and 4.6% suggesting that the 10× fields were relatively homogeneous throughout the section. Since the remainder of the sections appeared homogeneous, a single 10× field was subsequently chosen and digitally captured to represent the section.

2.5. Data analysis

Microelectrode data were analyzed from impalements maintained throughout the course of each experiment. AP characteristics measured in the study were: MDP, AP amplitude of phase 0 (APA), maximum upstroke velocity (V_{max}), potential at the peak of the plateau (Plateau), and APD to 30, 50 and 90% repolarization (APD_{30}, APD_{50} and APD_{90}, respectively).

Data are expressed as mean±S.E.M. The statistical techniques used were one-way or two-way analysis of variance for repeated or nonrepeated measures, with Bonferroni’s test when the F-value permitted [21]. Significance was determined at P<0.05.

3. Results

3.1. ECG data

ECG data for both groups of dogs are shown in Table 1. P-wave duration and PR interval were both longer in old animals. All other variables did not differ.

3.2. Action potential characteristics and response to pharmacologic agents

Two age-related differences were seen in the action potential contour: plateau potential was more negative and APD was longer in the old than the adult tissue. Representative experiments and summary data for APD and plateau amplitude are shown in Fig. 1. The differences in APD_{90} and plateau between the two groups were rate-dependent, and maximal at a cycle length of 2000 ms. APD_{50} did not differ between the groups, nor were significant differences in maximum diastolic potential (MDP), AP amplitude, and V_{max} seen at any cycle length. APD_{90} was shorter in old atria. The values are summarized in Table 2, at a cycle length of 1000 ms. That APD_{50} was equal at both ages while APD_{90} was longer in the elderly highlights the fact that the slope of phase 3 repolarization was more gradual in the old than in the adult tissues.

Fig. 2 depicts the actions of the $I_{Ca,L}$ agonist Bay K 8644 in adult and old atria. Qualitatively, the effects of the compound were similar in both tissues and consisted of an elevation of the plateau and shortening of AP duration (Fig. 2A). However, quantitative differences were clearly seen: Bay K 8644 elevated the plateau and shortened APD more in old than in adult tissue (Fig. 2B). Bay K 8644 effects were rate-dependent in both tissues and greatest at the longest cycle length (Fig. 2C). Interestingly, in the presence of Bay K 8644, the AP contour of the old atria approached that of adult atria superfused with control Tyrode’s solution. At 10^{-6} mol/l and a cycle length of 2000 ms, plateau potential (−6±1 mV) and APD_{90}

![Fig. 1. (A) Representative recordings from preparations of adult and old atria. In each panel, the top trace shows action potentials and the bottom, V_{max}. Vertical calibration is for action potentials and V_{max}; horizontal for action potentials. (B) Plateau potential and (C) action potential duration in preparations of adult and old atria during steady state stimulation at different cycle lengths; n=68 for adult and n=72 for old. APD_{90}, APD_{50}, and APD_{30}, action potential duration to 90, 50, and 30% repolarization, respectively. *P<0.05 versus adult at the same cycle length.](https://academic.oup.com/cardiovascres/article-abstract/54/2/462/275838)
Table 2
Transmembrane action potential characteristics recorded from adult and old atria at a cycle length of 1000 ms

<table>
<thead>
<tr>
<th>Group</th>
<th>MDP (mV)</th>
<th>APA (mV)</th>
<th>(V_{\text{max}}) (V/s)</th>
<th>Plateau (mV)</th>
<th>APD(_{30}) (ms)</th>
<th>APD(_{50}) (ms)</th>
<th>APD(_{90}) (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult</td>
<td>-79±1</td>
<td>104±1</td>
<td>199±7</td>
<td>-5±1</td>
<td>23±1</td>
<td>84±3</td>
<td>185±4</td>
</tr>
<tr>
<td>Old</td>
<td>-78±1</td>
<td>102±1</td>
<td>192±6</td>
<td>-13±1*</td>
<td>18±1*</td>
<td>77±3</td>
<td>205±3*</td>
</tr>
</tbody>
</table>

MDP, maximum diastolic potential; APA, action potential amplitude; APD\(_{30}\), APD\(_{50}\) and APD\(_{90}\), action potential duration to 30, 50 and 90% repolarization; \(V_{\text{max}}\), maximum upstroke velocity. Mean±S.E.M. are shown (\(n=68\) for adult and \(n=72\) for old). *\(P<0.05\) versus adult.

(193±6 ms) in old tissue did not significantly differ from the values for adult tissue in the absence of the compound (−9±2 mV and 195±8 ms, respectively). Bay K 8644 had no effects on MDP, AP amplitude and \(V_{\text{max}}\) in either tissue (data not shown).

Fig. 3 shows that the \(I_{\text{Ca,L}}\) antagonist nisoldipine had qualitatively different effects on adult and old atria. While not affecting the plateau in old tissue, nisoldipine concentration-dependently depressed the plateau in adult atria (Fig. 3B, upper panel). APD\(_{90}\) was concentration-dependently prolonged at all but the shortest cycle length in old dogs (Fig. 3B and 3C, lower panels) whereas in adults no significant change in APD\(_{90}\) was observed (Fig. 3B, lower panel and C, upper panel). At a cycle length of 2000 ms, the slope of phase 1 was 4.2±0.4 V/s in adult and 6.1±0.4 V/s in old tissue (\(P<0.05\)). Nisoldipine increased the slope in adult (to 6.1±0.5 V/s, \(P<0.05\)) but not in old (to 6.5±0.5 V/s, \(P>0.05\)) tissues. MDP, AP amplitude and \(V_{\text{max}}\) remained unchanged in the presence of nisoldipine in both groups (data not shown).

![Fig. 2](https://academic.oup.com/cardiovascres/article-abstract/54/2/462/275838)

*Fig. 2. Effects of Bay K 8644 (Bay K) on action potential in preparations of adult and old atria. (A) Representative experiments illustrating the effects of 10^−6 mol/l Bay K 8644 at a cycle length of 2000 ms (C=control). (B) Concentration-dependent effects of Bay K 8644 on plateau potential (upper panel) and APD\(_{90}\) (lower panel) at a cycle length of 2000 ms. \(P<0.05\) versus respective control. \(P<0.05\) versus adult at the same Bay K 8644 concentration. (C) Rate-dependent effects of Bay K 8644 (10^−6 mol/l) on APD\(_{90}\) in adult (upper panel) and old (lower panel) free wall atrial endocardium. \(P<0.05\) versus control at the same cycle length. In B and C, \(n=10\) for adult and \(n=16\) for old.*

![Fig. 3](https://academic.oup.com/cardiovascres/article-abstract/54/2/462/275838)

*Fig. 3. Effects of nisoldipine on action potential in preparations of adult and old atria. (A) Representative experiments illustrating the effects of 10^−6 mol/l nisoldipine at a cycle length of 2000 ms (C=control). (B) Concentration-dependent effects of nisoldipine on plateau potential (upper panel) and APD\(_{90}\) (lower panel) at a cycle length of 2000 ms. \(P<0.05\) versus respective control. \(P<0.05\) versus adult at the same nisoldipine concentration. (C) Rate-dependent effects of nisoldipine (10^−6 mol/l) on APD\(_{90}\) in adult (upper panel) and old (lower panel) atrial endocardium. \(P<0.05\) versus control at the same cycle length. In B and C, \(n=14\) for adult and \(n=10\) for old.*
3.3. Determinants of conduction

In the experiments on membrane responsiveness and conduction velocity, the values for $V_{\text{max}}$ did not differ between the groups (adult, $207\pm 17\ V/\text{s}$ and old $202\pm 18\ V/\text{s}$, $P>0.05$) whereas APD$_{90}$ (181±8 and 209±9 ms, respectively, $P<0.05$) and ERP (170±7 and 191±8 ms, respectively, $P<0.05$) were significantly longer in old tissue. Fig. 4A shows the relationship between membrane potential and $V_{\text{max}}$ in preparations from adult and old atria. There were no significant differences between the two curves: normalized $V_{\text{max}}$ was almost the same in both groups at all membrane potentials. However, when $V_{\text{max}}$ and conduction velocity were plotted against the diastolic interval, which was measured from the ERP to the onset of the next (extrasystolic) action potential, important differences between the groups were detected (Fig. 4B and 4C). Specifically, when the diastolic interval was decreased there was—over a wide range of intervals—a greater reduction in $V_{\text{max}}$ and a slower conduction velocity in old tissue. This was most marked at a zero diastolic interval (defining the ERP), at which conduction velocity had decreased 2.4-fold in adults versus 4.7-fold in the elderly.

3.4. Histological analysis

Representative sections from the atrium of an adult and old dog are shown in Fig. 5. The myocardial fibers appeared to be more compact and were closer together in adult compared with old tissue. In the sections of aged atria, muscle bundles were separated by large strands of connective tissue. A significantly higher deposition of connective tissue was found in sections of the aged atria: fibrosis was $4.8\pm 1.1\%$ ($n=12$) in adult versus $8.4\pm 1.0\%$ ($n=18$) in old ($P<0.05$).

4. Discussion

We have shown that certain characteristics of canine atrial AP change with aging. The most remarkable alteration is a significant lowering of the plateau. The major currents which determine the plateau level in canine atrium are $I_{\text{kur}}$ [22], $I_{\text{i}}$ [23] and $I_{\text{Ca-L}}$. Two mechanisms can account for the lower plateau in aged tissue in comparison to adults: an increase in repolarizing currents ($I_{\text{kur}}$ and/or $I_{\text{w}}$) or a decrease in depolarizing current $I_{\text{Ca-L}}$. The results with nisoldipine and Bay K 8644 suggest the latter is a likely mechanism for the following reasons: First, the $I_{\text{Ca-L}}$ blocker nisoldipine markedly lowered the plateau in adults but had no effect on plateau height in old atria whereas the $I_{\text{Ca-L}}$ agonist Bay K 8644 elevated the plateau more prominently in old tissue. This is consistent with a lesser $I_{\text{Ca}}$ in aged tissue. Second, nisoldipine sped phase I repolarization in adult with no effect in old atria. As a
Fig. 5. Representative sections of pectinate muscle bundles from an adult (A) and old (B) dog showing myocytes in red and collagen in blue. The circular, clear spaces are interstitial fat which was excluded from the analysis. Image analysis of these and additional fields showed the amount of fibrosis tissue in adult atrium = 4.5% (A) and the amount of diffuse, interstitial fibrous tissue in old atrium = 17.9% (B) of the amount of myocardium. Masson trichrome stain. Original magnification = 160×.
result, nisoldipine eliminated the difference in the slope of phase 1 between adult and old atria. The equalization of phase 1 slope by nisoldipine (i.e. in conditions of $I_{Ca}$ inhibition) suggests the decrease in $I_{Ca}$ as a major mechanism for the low plateau in aged tissue. However, alterations in repolarizing currents which have been detected in aged canine atria [24] can also contribute to the difference in plateau level between two tissues.

The longer APD$_{90}$ in old atria suggests some aging-induced changes of a delayed rectifier potassium current ($I_k$) or may be simply a consequence of the low plateau level in this tissue. Both the activation time constant and amplitude of $I_k$ in atrial myocytes are voltage-dependent [23,25]. A low plateau potential in aged tissue may slow activation of $I_k$ and prolong the final phase of repolarization. Accordingly, plateau elevation induced by Bay K 8644 results in APD shortening. Interestingly, in the presence of Bay K 8644, the action potential contour in aged atria approached that of the adult in the absence of the compounds.

The most important findings of the present study relate to the conduction of premature stimuli in old and adult atria. Aged tissue showed a significantly greater tendency to slow propagation, both in terms of a decrease in conduction velocity and of a wider time zone during which the early premature impulses were slowly conducted. Conduction velocity is determined by the membrane properties of each cell and electrical coupling among cells [26]. No difference in the dependence of $V_{max}$ on membrane potential between adult and old tissues was detected in the present study. The results suggest that the function of the fast sodium channels is similar in both tissues and the slowing of conduction in old atria is induced by changes in other active and/or passive properties.

It has been demonstrated that age-related changes in the content and distribution of connective tissue reduce the degree of cellular coupling and lead to discontinuous propagation (slow conduction across increased junctional resistance) both in atria [27] and in ventricles [28]. Moreover, under normal conditions, enough depolarizing current is transferred across such discontinuities to maintain normal propagation. However, when the maximum membrane depolarizing current is reduced by premature stimulation, conduction slowing and even block can occur [29]. Our data are compatible with these findings. First, we found that in old atria, the myocardial fibers were less compacted and the content of fibrous tissue was significantly higher than in adults. Second, we found no significant difference between the two groups in conduction of normal beats but significantly slower conduction of early premature impulses in aged atrial tissue. In addition, the important role of $I_{Ca-L}$ in the maintenance of discontinuous conduction has been demonstrated in theoretical and experimental studies [30–32]. The driving force for discontinuous conduction is determined by the plateau potential and $I_{Ca-L}$ becomes the major current source maintaining conductance [30,31]. We found more negative plateau potentials in old atria which implies a lower driving force for conduction of early premature beats in the old than in the adult tissue. Thus, our results suggest that the age-related structural changes in the atrial myocardium as well as alterations in the AP contour are responsible for a reduced conduction of premature beats in old atria. The wider window of diastolic intervals with a reduced $V_{max}$ and conduction velocity in aged tissue reflects most likely more gradual and longer phase 3 repolarization in old atria.

P-wave duration was significantly longer in the old than the adult dogs. The P-wave duration, which correlates well with intra-atrial conduction time [33], depends mainly on conduction velocity and atrial size [34]. We found that conduction velocity of normal beats was slightly lower in old atria. Thus, the longer P-wave duration in the old dogs might be a reflection of aging-associated atrial enlargement [11] and some degree of reduced conduction.

The implications of the present findings relate to the mechanisms of the increased susceptibility of aged atria to atrial fibrillation. In canine and goat models of atrial fibrillation the primary change that results from rapid pacing and that facilitates fibrillation is acceleration of repolarization and, with this, shortening of the ERP. Abnormalities of conduction are not prominent in these models. To our knowledge, all studies of pacing-induced atrial fibrillation have been carried out in adult animals. Yet, fibrillation is typically a disease of the elderly in dogs as well as humans. Hence, it would be reasonable to suggest that the substrate found in the normal old heart may be more conducive to fibrillation than that in adults. Our studies appear to suggest that this is the case. It appears that conduction changes are a normal accompaniment of aging as is seen in the longer P-wave duration on ECG in both human subjects and dogs. As shown in the present study, conduction slowing is especially prominent for premature impulses arising during phase 3 repolarization in the elderly. This type of conduction slowing would appear most applicable to premature atrial depolarizations, increasing the likelihood of slow conduction and possibly reentry. It is this type of propagation that might well further facilitate the onset of fibrillation.

A correlation between prolongation of atrial activation time and incidence of atrial arrhythmias has long been recognized. For example, slow conduction of early premature impulses has been found in patients with paroxysmal atrial fibrillation [33,35]. Moreover, patients with paroxysmal atrial fibrillation show longer delay zones and maximum delays than patients without atrial arrhythmias, while no significant difference may be found in basal conduction [36,37]. Interestingly, the longest coupling interval giving rise to interatrial conduction delay showed a significant correlation with age in patients with no documented episodes of paroxysmal atrial fibrillation [38]. The age-related changes in conduction velocity observed in the present study (reduced conduction velocity and wider time window with slow conduction in aged atria in comparison to adult) are consistent with the above clinical
data and may therefore explain the greater vulnerability to atrial fibrillation in the elderly.

Acknowledgements

The authors express their gratitude to Dr Natalia Egorova for assisting in the performance of the experiments. We also thank Eileen Franey for her careful attention to the preparation of the manuscript. This study was supported by USPHS-NHLBI grant HL-67449.

References