Differences in the Aging-Associated Trends of the Monophasic Action Potential Duration and Effective Refractory Period of the Right and Left Atria of the Rat

Congxin Huang, MD; Wenmao Ding, MD; Lan Li, MD*; Dongdong Zhao, MD

Background  The incidence of atrial fibrillation (AF) increases with aging, but the aging-associated electrophysiological changes of atrial myocardium are poorly understood.

Methods and Results  Based on the hypothesis that aging of the atrium enhances AF susceptibility, 30 Wistar rats were divided into 3 age groups: adult, middle-aged, and aged (n=20 per group). Their hearts were isolated and perfused by Langendorff apparatus. Monophasic action potential duration at 90% repolarization (MAPD₉₀) and effective refractory period (ERP) at the basic stimulation cycle length (BCL: 400 ms), and MAPD₉₀ at other different stimulation cycle lengths in each age group were measured. At the BCL, the MAPD₉₀ of the right atrial myocardium was prolonged from the adult to the aged group, that of the left atrial myocardium was prolonged from the adult to middle-aged group, and the MAPD₉₀ of the left atrial myocardium in the aged group were shorter than that in the adult and middle-aged groups. The ERP of the atrial myocardium showed the same age-associated trend as MAPD₉₀. As the stimulation frequency increased, the MAPD₉₀ of both the left and right atrial myocardium shortened correspondingly in the adult and middle-aged groups, but in the aged group the MAPD₉₀ of the right atrial myocardium shortened markedly more than that of the left atrial myocardium.

Conclusions  There are different aging-associated electrophysiological changes in the right and left atrium, and the older heart is more vulnerable to developing the substrate for AF. (Circ J 2006; 70: 352–357)

Key Words:  Aging; Atrium; Effective refractory period; Electrophysiology; Monophasic action potential

Elderly people are prone to various cardiac arrhythmias, of which atrial fibrillation (AF) is the most common, and epidemiological research has shown the close relationship between the incidence of AF and aging.1,2 AF usually occurs in conjunction with other cardiovascular diseases, such as coronary heart disease, rheumatic heart disease, and chronic heart disease, but not all patients with AF have an underlying disease,3 which suggests that aging-associated changes of atrium itself may play an important role in the mechanisms of AF.

There is growing awareness that the atrium has substrates that are vulnerable to the development of AF.1–6 Although aging is a normal physiological process, it alters the electrophysiological features of the cardiac myocytes, such as the membrane currents (ie, Iᵣ, Iᵣ₋₅₋₁, Na⁺/K⁺ pump) and the action potential duration (APD) of ventricular myocytes.7–10 However, the aging-related electrophysiological changes of the atrial myocardium are poorly understood.

Previous studies have also shown that there are differences between the right and left atria in refractoriness and APD,11–15 which may be an important physiological factor in the maintenance of AF, and the effect of aging on these differences also needs to be further studied.

Our hypotheses for the present study were that (1) aging affects the atrial electrophysiological features and (2) the aged heart could provide the substrate for AF. Shortening of the monophasic APD (MAPD) and maladaptation of the MAPD to rate are 2 important features of AF. Consequently, the aims of the present study were to investigate the effects of aging on the monophasic action potential (MAP) and effective refractory period (ERP) of rat atrial myocardium and the rate-related change in MAPs.

Methods  All experiments conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication, revised 1996).

Animals  Healthy male Wistar rats in 3 age groups [adult (7–8 month of age, 350–400 g body weight, n=20), middle-aged (13–15 month of age, 450–500 g body weight, n=20) and aged (over 2 year of age, 550–650 g body weight, n=20)] were obtained from the experimental animal center of Wuhan University. All animals were maintained on a 12:12 h light–dark cycle and allowed free access to food and water for at least 1 month before being this study.

Solutions  The pH of Krebs-Henseleit solution ([mmol/L]: NaCl 140, KCl 5.4, MgCl₂·6H₂O 1.0, CaCl₂·2H₂O 1.8, glucose 5.0, sodium pyruvate 4.0, tris base 10) was adjusted to 7.4 with HCl, and maintained by equilibration with 5%CO₂–95%O₂. All reagents were of analytical purity grade.
Heart Preparation

The entire hearts were carefully excised from anaesthetized rats (150 mg/kg pentobarbitone ip), and rapidly trimmed in ice-cold 0.9% NaCl. The aorta was cannulated immediately, then rapidly transferred to a vertical Langendorff apparatus and perfused with oxygenated Krebs-Henseleit solution at a constant perfusion pressure of 73 mmHg using roller pumps and at a constant temperature of 37°C. The entire heart was submerged in a bath of perfusate at 37°C. All hearts were Langendorff-perfused for 20 min before recording.

Recording of MAP and ERP

The recording electrode used in the study was a machine-pulled standard capillary electrode filled with 3 mol/L KCl. The bipolar stimulation electrode and the reference electrode were made of teflon-coated stainless steel wire, the distal tip of which was 0.5 mm long and naked.

MAPs were obtained from the epicardium of the right and left atria. The stimulation electrode was fixed to the epicardial surface of the right ventricle, and the reference electrode to the root of the pulmonary artery. The recording electrode position was regulated by a mini manipulator (Olympus) to ensure its tip penetrated into the center of the free wall of the atrium. MAPs were considered acceptable when at least 5 consecutive MAPs were identical and clear. MAPD90 was the average value of 3 consecutive MAPs at 90% repolarization, and MAPD20-50 was the mean value of 3 consecutive MAPs from 20-50% repolarization. MAPD90 and MAPD20-50 were measured at stimulation cycle lengths of 400 ms, 350 ms, 300 ms and 250 ms, with 400 ms defined as the basic cycle length (BCL). Stimulation impulse signals were generated by an electrophysiological stimulator (Suzhou) at twice the diastolic threshold, which were generated by a constant voltage stimulator (Suzhou) and following a train of 8 regular stimuli (S1) at the BCL, an extrastimulus (S2) was introduced and then subsequently introduced progressively in 5-ms steps until it failed to trigger an action potential. ERP was defined as the longest S1S2 interval at which S2 failed to produce a propagated atrial response. The determination of the ERP in each age group was completed within 2 min.

The MAP and ERP were recorded at 5 different sites within a selected region of both atria in each heart. Perfusate pH, bath temperature, and the recording electrode were checked before each set of recordings.

Statistical Analysis

All data are presented as means ± standard deviation. Statistical analysis was performed using 1-way analysis of variance (ANOVA) and Student-Newman-Keuls test (q-test) when 2 parameters were compared within the group. Differences with a p-value <0.05 were considered significant.

Results

The MAPs at different stimulation cycle lengths in each age group are shown in Fig 1.

Effect of Aging on the MAPD90 of Atrial Myocardium

At the BCL, the MAPD90 of the right atrial myocardium of the adult, middle-aged and aged groups was 125±9 ms, 143±11 ms, and 146±18 ms, respectively (p<0.01). The MAPD90 of the left atrial myocardium was prolonged from the adult group (120±6 ms) to the middle-aged group (136±9 ms) (p<0.05, q-test), and that of the aged group (105±13 ms) was much shorter than in the other 2 groups (p<0.05)

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respectively, q-test).

In the adult and middle-aged groups the MAPD90 of the right atrial myocardium was similar to that of the left atrial myocardium (both NS), but in the aged group the MAPD90 of the right atrial myocardium was significantly longer than that of the left atrial myocardium (p<0.01) (Fig 2).

**Effect of Aging on the MAPD20–50 of Atrial Myocardium**

At the BCL, the MAPD20–50 of the right atrial myocardium of the adult, middle-aged and aged groups was 24±6 ms, 28±7 ms, and 31±10 ms, respectively (NS), and that of the left atrial myocardium was 20±5 ms, 26±6 ms, and 21±7 ms, respectively (NS).

In adult and middle-aged groups the MAPD20–50 of the right atrial myocardium was similar to that of the left atrial myocardium (both NS), but was significantly longer in the aged group (p<0.05).

### Table 1 MAPD90 of the Atrial Myocardium in Each Age Group at Different Stimulation Cycle Lengths

<table>
<thead>
<tr>
<th>Cycle length (ms)</th>
<th>Right atrium</th>
<th>Left atrium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adult group</td>
<td>Middle-aged group</td>
</tr>
<tr>
<td>400</td>
<td>125±9</td>
<td>143±11</td>
</tr>
<tr>
<td>350</td>
<td>120±6</td>
<td>137±5</td>
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<tr>
<td>300</td>
<td>113±5</td>
<td>127±10</td>
</tr>
<tr>
<td>250</td>
<td>99±6*</td>
<td>109±8*</td>
</tr>
</tbody>
</table>

Values are means ±SD, n=20. MAPD90, monophasic action potential duration at 90% repolarization. *p<0.05 among these 4 stimulation cycle lengths in the same age group, **p<0.01 vs right atrial myocardium at the same age group and at the same cycle length.

### Table 2 MAPD20–50 of Atrial Myocardial in Each Age Group at Different Stimulation Cycle Lengths

<table>
<thead>
<tr>
<th>Cycle length (ms)</th>
<th>Right atrium</th>
<th>Left atrium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adult group</td>
<td>Middle-aged group</td>
</tr>
<tr>
<td>400</td>
<td>24±6</td>
<td>28±7</td>
</tr>
<tr>
<td>350</td>
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<td>300</td>
<td>18±5</td>
<td>22±5</td>
</tr>
<tr>
<td>250</td>
<td>14±3*</td>
<td>17±4*</td>
</tr>
</tbody>
</table>

Values are means ±SD, n=20. MAPD20–50, monophasic action potential duration from 20% to 50% repolarization. *p<0.05 among the different stimulation cycle lengths, **p<0.01 vs right atrial myocardium at the same age group and at the same cycle length.

**Effect of Aging on the ERP of Atrial Myocardium**

The ERP of the right atrial myocardium of the adult, middle-aged and aged groups was 75±6 ms, 85±6 ms, and 86±4 ms, respectively (p<0.01). The ERP of the left atrial myocardium increased only gradually from the adult group (74±3 ms) to the middle-aged group (84±5 ms) (p<0.05, q-test), but in the aged group it was shorter than in the other 2 groups (68±6 ms) (p<0.05 respectively, q-test).

There was no difference between the right and left atrial myocardium in the adult and middle-aged groups for ERP (NS, respectively), but there was a significant difference in the aged group (p<0.01) (Fig 3).

**Effect of Stimulation Cycle Length on the MAPD90 of Atrial Myocardium**

Tables 1 and 2 show the MAPD90 of each age group at different stimulation cycle lengths, and Figs 4 and 5 show the change in the MAPD90 and MAPD20–50 of the atrial myo-
cardium with shortening of the stimulation cycle length.

In each age group the MAPD\textsubscript{90} shortened as the stimulation frequency increased. In the middle-aged and adult groups, the MAPD\textsubscript{90} of the right and left atrial myocardium changed correspondingly, but in the aged group as the stimulation frequency increased, the MAPD\textsubscript{90} of the right myocardium shortened markedly and the left atrial myocardium did not show the same trend.

The MAPD\textsubscript{20–50} also shortened as the stimulation frequency increased. In the right atrium of the aged group there was a sharp change, but not in the left atrium in which the shortening trend of MAPD\textsubscript{20–50} was flat.

**Discussion**

The MAP vividly reflects the duration and configuration of the transmembrane action potential, and recording techniques bridge the gap between cellular and clinical cardiac electrophysiology. In the present experiment we used a glass microelectrode as the recording electrode because research\textsuperscript{8,19} has shown that it reflects the AP of the cardiac myocyte, and compared with the results from the recording electrode used in our previous study\textsuperscript{17} MAPs were easily obtained using the glass microelectrode and their amplitude was much higher. The MAP duration obtained by the 2 types of recording electrodes were identical.

There are 2 main findings of the present study. Both the MAPD\textsubscript{90} and ERP of the left and right atrial myocardium have different trends with changes in age. In the aged group the MAPD\textsubscript{90} and ERP of the left atrial myocardium were significantly shorter than those of the right atrial myocardium. Second, as the stimulation frequency increases, the changes in MAPD\textsubscript{90} and MAPD\textsubscript{20–50} of the right atrial myocardium were not similar to those of the left atrial myocardium in the aged group. The shortening of the MAPD\textsubscript{90} and MAPD\textsubscript{20–50} of the left atrial myocardium was less than in the right atrium.

Previous studies have shown that the membrane ion currents of aged myocardial cells differ from those of the young heart.\textsuperscript{7,8} The main changes are increasing I\textsubscript{to}, decreasing I\textsubscript{Ca,L} with slower inactivation, and prolonged APD of the myocyte. Another study also showed that the AP of the aged myocardial cell was prolonged in order to maintain the balance of the ion components, especially the cellular Ca\textsuperscript{2+}.\textsuperscript{9} In fact, the membrane ion channel is in a compensation state to maintain the cellular ion homeostasis. Those findings are consistent with most of the results of our present study.

However, our observation that the MAPD\textsubscript{90} and ERP of the left atrium shortened in the aged group deviated from this pattern, by an unknown mechanism, although there are 2 possibilities.

The first cause may be dilation of the atrium, which can lead to shortening of the MAPD\textsubscript{90} and ERP. Another factor is activation of a volume-sensitive ion current that is an outward potassium ion current\textsuperscript{20} or a decrease in all the membrane ion currents, with the calcium ion current decreasing most\textsuperscript{21}. Under physiological conditions, the load, volume and pressure of the left atrium increase with advancing age, and this change will be more marked in the aged heart. In addition, the I\textsubscript{Ca,L} of the cardiac myocyte in the aged group is in a state of compensation, which will make I\textsubscript{Ca,L} decrease markedly.

The second cause might involve the cardiac myocyte itself. Aging is characterized by a progressive deterioration in physiological functions and metabolic processes. The concentration of free calcium ions is increased in the aged myocardial cell and to decrease that concentration, the
myocardial cell might decrease ICa,L. So a radical cause of the different changes in old atrium might be the decrease in ICa,L. Previous studies have shown that decreasing of ICa,L is an important cause of maladaptation of MAPD to rate. It and in the present study, the MAPD20-50 of the left atrium in the aged group was shorter than that of the right atrium, and also shorter than that of the left atrium in the adult group. In addition, the shortening ability of the MAPD90 and MAPD20-50 of the left atrium of the aged group in relation to the stimulation cycle length was much less than that of the right atrium, which would be consistent with the foregoing explanations.

I0 and ICa,L are the main membrane ion currents that affect APD. With I0 increasing and ICa,L decreasing, the MAPD90 and ERP of the left atrial myocardium became shorter in the aged group than in the other 2 groups or in the right atrial myocardium. The shorter ERP would make the rat atrial myocardium separate early from the refractory period and quickly restore excitability, which would make the atrial tissue able to receive a high frequency stimulation and ectopic beat. The shorter MAPD90 would make the re-entrant wavelength become shorter in the atrium and thus a re-entrant ring would become more stable. These are important mechanisms for the initiation and development of AF.

Evidence suggests that reentry mechanisms are responsible for the genesis of AF. Heterogeneity of the MAP between the left and right atria can create the substrate for re-entrant arrhythmias and thus vulnerability to fibrillation. It has also been shown that the MAP and ERP of the right atrium are longer than those of the right atrium. In the present study, although the MAPD90 and ERP of the right atrial myocardium were longer than those of the left atrium, there were no significant differences between the adult and middle-aged groups. The difference may lie in the animal model or experimental method. However, in the aged group there were significant differences between the right and left atrial myocardium in MAPD90 and ERP, which can be explained by the conclusions reached earlier. The 2 atria will be uncoordinated in their repolarization and therefore their excitability and refractoriness. Should a fast ectopic atrial premature beat occur under such conditions, a re-entrant circuit will be easily formed.

Other studies have attached particular importance to the left atrium in the initiation and maintaining of AF and the present study showed that there were aging-associated changes of the electrophysiological properties of the atrial myocardium. In the aged group there was a shorter MAPD90 and ERP of the left atrial myocardium, the shortening ability of the MAPD90 in relation to an increase in the simulation frequency was not maintained in the left atrial myocardium, there were differences between the left and right atrial myocardium, and so on, which we consider would provide the substrate for AF.

Study Limitations

The isolated heart preparation is devoid of autonomic and hemodynamic influences, which may equalize or enhance the disparities in the MAP between the left and right atria. Compared with previous studies, the dispersion of our study was small, and the discrepancy may lie in the animal model and/or study method. Although our study showed that there are aging-associated electrophysiological changes in the rat atrium, it remains to be determined how much these effects can be applied to the clinical situation.

References

