Effects of Aging and Ouabain on Left Atrial Arrhythmogenicity

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Left Atrial Arrhythmogenicity and Aging. ***Objectives:*** **Aging increases atrial fibrillation (AF) vulnerability. The left atrium (LA) is important for the generation of AF. However, the effect of aging on the electrophysiological properties of the LA in general, on the specific LA sites, and of possible accentuation of regional differences between the LA sites with aging is not clear. The purpose of this study was to evaluate the effects of aging on the LA electrophysiological heterogeneity and ouabain-induced arrhythmogenicity.**

Methods: **We used conventional microelectrodes to record the action potentials (APs) in isolated young (age, 3 months) and aged (age, 3 years) rabbit LA posterior wall (LAPW) and LA appendage (LAA) tissue specimens before and after the administration of ouabain.**

Results: **Young LAPWs ($n = 10$) had larger AP amplitudes than young LAAs ($n = 10$, $P < 0.05$), and aged LAPWs ($n = 9$) had longer AP durations than aged LAAs ($n = 9$, $P < 0.05$). Ouabain (1 μM) induced a higher incidence (80% vs 30%, $P < 0.05$) of delayed afterdepolarizations (DADs) and spontaneous activity (60% vs 10%, $P < 0.05$) in the young LAPWs than in the young LAAs. Compared with the young group, the aged LAs had a higher incidence of DADs with a less negative resting membrane potential and smaller maximum upstroke velocity. After the ouabain (1μM) administration, the aged LAPWs had a greater shortening of the AP duration. Ouabain-induced spontaneous activity was similar between the young and aged groups.**

Conclusions: **Aging enhanced the LA regional electrical heterogeneity and LAPW arrhythmogenesis.**
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aging, atrial fibrillation, glycosides, heterogeneity, left atrium

Introduction

Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia with significant morbidity and mortality.[1] Previous studies have shown that the left atrium (LA) is important in the AF pathyphysiology.[2,3] Several investigators have shown the regional differences in LA electrophysiologic properties.[4-7] The LA posterior wall (LAPW) exhibited a significantly shorter AF cycle length, with frequent rapid and repetitive electrical discharges than that of the other LA regions.[4-6] The significantly higher incidence of ectopic beat-initiating AF from the LAPW suggests the arrhythmogenic potential of the LAPW.[7] However, limited information has been available regarding the electrophysiology of the LAPW. A heterogeneous atrial electrophysiology is known to increase the atrial dispersion and facilitate the occurrence of AF. It has been shown that the right atrium (RA) contains an electrophysiological heterogeneity and may result in the genesis of atrial arrhythmias.[8] Therefore, it is possible that a regional electrophysiologic difference in the LA may play a role in the arrhythmogenic mechanism of AF.

Aging is an important factor for the AF pathophysiology and the prevalence of AF increases with age.[1] However, the mechanism of increasing AF vulnerability in aging remains poorly understood. Aging has been shown to increase the atrial interstitial fibrosis, decrease the conduction velocity, and change the action potential (AP) characteristics, which may facilitate the occurrence of AF.[9-12] Whether aging may increase the LAPW arrhythmogenic activity to cause AF is still unknown. Cardiac glycosides that are frequently used in treating heart failure, especially for the rate control of AF, can aggravate tachycardia-induced atrial electrical remodeling with a AF predisposition.[13,14] Nevertheless, the knowledge of digitalis effects in age and regional differences is unclear. Our previous study has shown the different ouabain effects between those on the pulmonary veins and those on the LA.[15] Thus, the ouabain's effects may differ in the various LA regions to cause different arrhythmogenesis. The purpose of this study was to evaluate the effects of aging on the LA electrophysiological heterogeneity and ouabain-induced arrhythmogenicity.

Methods

Rabbit LA Tissue Preparations

The investigation conformed to the institutional *Guide for the Care and Use of Laboratory Animals.* Young rabbits

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(3 months) and aged rabbits (3 years) were anesthetized with an intraperitoneal injection of sodium pentobarbital (40 mg/kg). A mid-line thoracotomy was then performed and the heart and lungs were removed. For the dissection of the LAPW and left atrial appendage (LAA), the LA was opened by an incision along the mitral valve annulus, extending from the coronary sinus to the septum in a Tyrode's soluton with a composition (in mM) of 137 NaCl, 4 KCl, 15 $NaHCO_3$, 0.5 NaH_2PO_4, 0.5 $MgCl_2$, 2.7 $CaCl_2$, and 11 dextrose. The LA was separated from the pulmonary veins at the LA-pulmonary vein junction. Tissue strips ($\sim$10 $\times$ 5 $\times$ 0.5 mm) from the LAPW and LAA were then dissected. One end of the preparation was pinned with needles to the bottom of a tissue bath. The other end was connected to a Grass FT03C force transducer with a silk thread. The tissue was superfused at a constant rate (3 mL/min) with Tyrode's solution that was saturated with a 97% O_2–3% CO_2 gas mixture. The temperature was maintained constant at 37°C and the preparations were allowed to equilibrate for 1 hour before the EP study. To assess the preparation viability, any tissues with a resting membrane potential (RMP) less than –65 mV before drug administration were excluded from the study and the contractile force elicited at a 2-Hz electrical stimulus had to be obtained throughout the entire study protocol to ensure the viability. The electrophysiologic recordings from the superfused preparations remained stable and viable for an experimental period of 2 hours.

Electrophysiological and Pharmacological Studies

The transmembrane AP of the LAPW and LAA were recorded by means of machine-pulled glass capillary microelectrodes filled with 3M of KCl, and the LA preparation was connected to a WPI model FD223 electrometer under tension with 150 mg. The electrical and mechanical events were displayed simultaneously on a Gould 4072 oscilloscope and Gould TA11 recorder. The signals were recorded with DC coupling and a 10-KHz low-pass filter cutoff frequency using a data acquisition system. Signals were recorded digitally with 16-bit accuracy at a rate of 125 KHz. An electrical stimulus with a 10-ms duration and suprathreshold strength (30% above the threshold) was provided by a Grass S88 stimulator through a Grass SIU5B stimulus isolation unit. Different concentrations of ouabain (0.1, 1 μM) were sequentially superfused to test the pharmacological responses. The LAPW and LAA preparations were treated with ouabain for at least 20 minutes for each concentration. The APs were elicited through a 2-Hz electrical stimulus before and after the drug administration. The RMP was measured during the period between the last repolarization and onset of the subsequent AP. The amplitude of the AP (APA) was obtained from the RMP to the peak of the AP depolarization. The AP duration at a repolarization of 90%, 50%, and 20% of the AP amplitude were measured as the APD_{90}, APD_{50}, and APD_{20}, respectively. The maximum upstroke velocity (V_{max}) was acquired by the maximum positive value of the first derivative of the AP. A delayed afterdepolarization (DAD) was defined as the presence of a spontaneous depolarization of the impulse after full repolarization had occurred. Spontaneous activity was defined as a constant occurrence of spontaneous APs without using any electrical stimuli.

Statistical Analysis

All quantitative data are expressed as the mean $\pm$ SEM. A paired *t*-test was used to compare the differences before and after the drug administration and the differences between LAPW and LAA within the same age group. A two-way analysis of variance was used to compare the differences between the young and aged LAPWs or LAAs. Multiple comparisons were analyzed with the Tukey test. Nominal variables were compared by a chi-square analysis with Yates correction or Fisher's exact test. A P value less than 0.05 was considered statistically significant.

Results

Electrophysiological Heterogeneity of the Young and Aged LA

As the examples show in Figure 1, there were only electric-driven APs without any spontaneous activity or DADs in the young LA. The amplitude of the AP (APA) was significantly larger in the young LAPW than in the young LAA (Table 1). However, the RMP, V_{max}, APD_{20}, APD_{50}, and APD_{90} were similar between the young LAPWs and LAAs.

In the aged LA, there were only electric-driven APs without any spontaneous activity (Fig. 1). The APD_{50} and APD_{90} in the LAPW were longer than those in the LAA (Table 1). In addition, three (33%)0 LAPWs and one (11%) LAA had

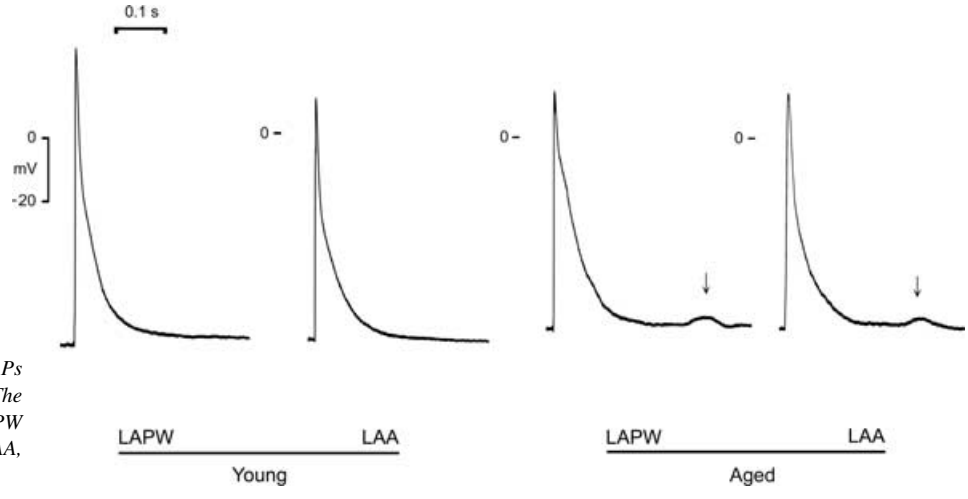

Figure 1. *Tracings showing examples of APs from young and aged LAPWs and LAAs. The DADs (↓) were recorded in the aged LAPW and LAA, but not in the young LAPW and LAA, with a 2-Hz electrical stimulus.*

TABLE 1
Baseline Electrophysiological Characteristics of Aged and Young LAPWs and LAAs

	Young		Aged	
Electrophysiological Properties	**LAPW (n = 10)**	**LAA (n = 10)**	**LAPW (n = 9)**	**LAA (n = 9)**
APA (mV)	97 ± 2	87 ± 3*	80 ± 3†	81 ± 3
RMP (–mV)	75 ± 1	75 ± 1	72 ± 1†	72 ± 1†
V_{max} (m/s)	125 ± 10	119 ± 9	80 ± 14†	85 ± 10†
APD_{20} (ms)	10 ± 1	8 ± 1	11 ± 2	9 ± 1
APD_{50} (ms)	23 ± 1	22 ± 1	26 ± 3	19 ± 2*
APD_{90} (ms)	81 ± 3	81 ± 3	95 ± 6†	77 ± 2*
ΔAPD_{90} (LAPW-LAA, ms)	7 ± 1		19 ± 3†	

*P < 0.05 versus the LAPW in the same age group.
†P < 0.05 versus the same region in the young LA.

DADs. However, the APA, RMP, V_{max}, and APD_{20} were similar between the two regions.

Compared with the young LA, both the aged LAA and LAPW had a less negative RMP and smaller V_{max}. In addition, the aged LAPW had a smaller APA and longer APD_{90} than the young LAPW (Table 1). In contrast, there were similar APA and APD_{90} between the aged and young LAAs. Moreover, the APD_{20} and APD_{50} of LAA and LAPW were similar between the young and aged LAs, respectively.

The incidence of DADs was studied in 19 young LAPWs, 15 young LAAs, and nine aged LAPWs and LAAs. DADs were observed in three of nine (33%) aged LAPWs, but in none of the 19 (0%) young LAPWs (P < 0.05). DADs were observed in only one out of nine (11%) aged LAAs and in none of the 15 (0%) young LAAs (P > 0.05).

Ouabain Effects on the Heterogeneity of the Young LA

As the examples and average data show in Figures 2 and 3, ouabain (0.1 μM) significantly decreased the RMP and shortened the APD_{90} in the LAA (n = 10) and LAPW (n = 10). Ouabain (0.1 μM) induced the occurrence of DADs in 40% of the LAPWs and in 20% of the LAAs. The amplitude of the ouabain-induced DADs (2.8 ± 0.1 mV vs 2.2 ± 0.1 mV, P > 0.05) was similar for the LAPW and LAA. However, ouabain (0.1 μM) only shortened the APD_{20} and APD_{50} in the LAPW, but not in the young LAAs.

Ouabain (1 μM) decreased the APA, RMP, and V_{max}, and shortened the APD_{20}, APD_{50}, and APD_{90} in both the LAPW and LAA. Ouabain (1 μM) induced the occurrence of DADs in 80% of the LAPWs (n = 10) and 30% of the LAAs (n = 10) (P < 0.05), and exhibited a significantly larger amplitude (5.0 ± 0.6 mV vs 2.4 ± 0.2 mV, P < 0.05) of the DADs in the LAPW than in the LAA. Ouabain (1 μM) also induced spontaneous activity (Fig. 2B) in six of 10 LAPWs with a duration of 4 ± 0.8 minutes, but only induced spontaneous activity in one of 10 LAAs with a duration of 1 minute (60% vs 10%, P < 0.05).

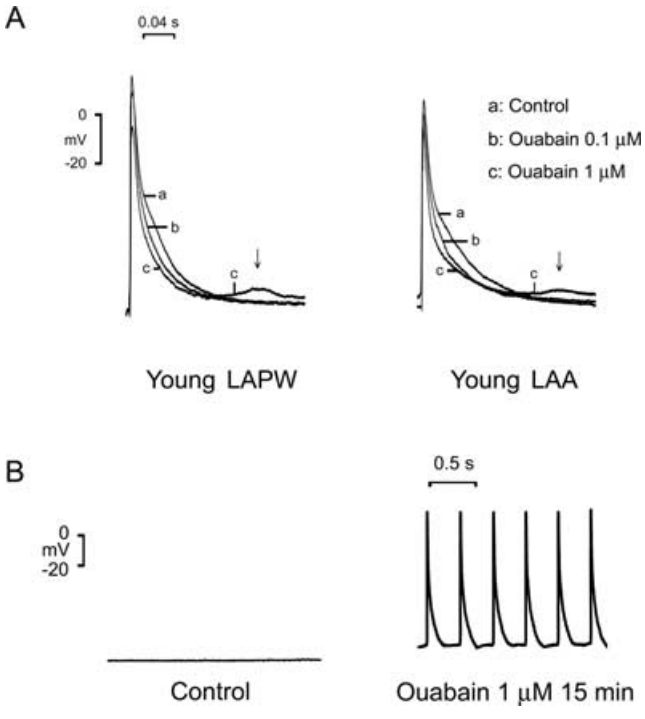

Figure 2. *Effects of ouabain on the young LAPWs and LAAs. Panel A: The superimposed tracings of the AP configuration before and after the ouabain (0.1, 1 μM) with a 2-Hz electrical stimulus. Note that ouabain (1 μM) induced the occurrence of a DAD (↓). Panel B: The tracing shows an example of ouabain (1 μM)-induced spontaneous activity in a young LAPW. The development of a phase 4 depolarization is noted.*

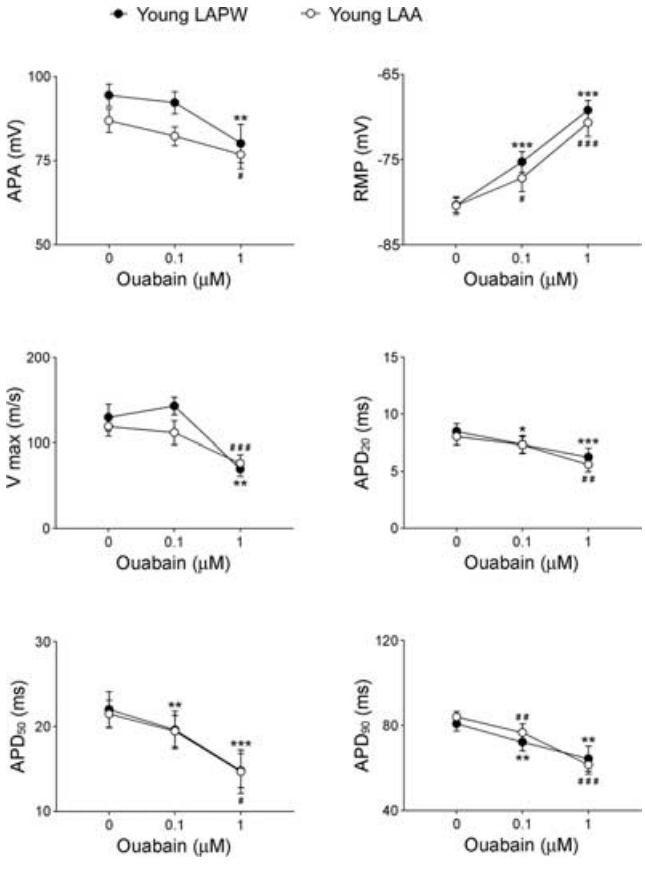

Figure 3. *The average data of the APA, RMP, V_{max}, APD_{20}, APD_{50}, and APD_{90} from the young LAPWs and LAAs before and after ouabain (0.1, 1 μM) administration measured during a 2-Hz electrical stimulus. *P < 0.05, and **P < 0.01, ***P < 0.001, comparsions between before and after ouabain (0.1, 1 μM) administration in LAPWs. #P < 0.05, and ##P < 0.01, ###P < 0.001, comparsions between before and after ouabain (0.1, 1 μM) administration in LAAs.*

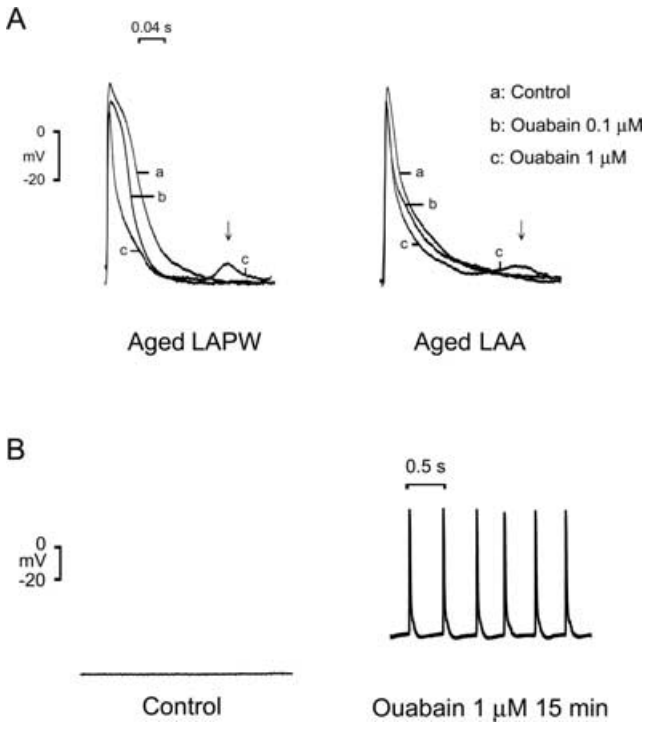

Figure 4. *Effects of ouabain on aged LAPWs and LAAs. Panel A: The superimposed tracings show the effects of ouabain (0.1, 1 μM) on the AP configuration with a 2-Hz electrical stimulus. Note that ouabain (1 μM) induced a DAD (↓). Panel B: The tracing shows an example of ouabain (1 μM)-induced spontaneous firing in an aged LAPW.*

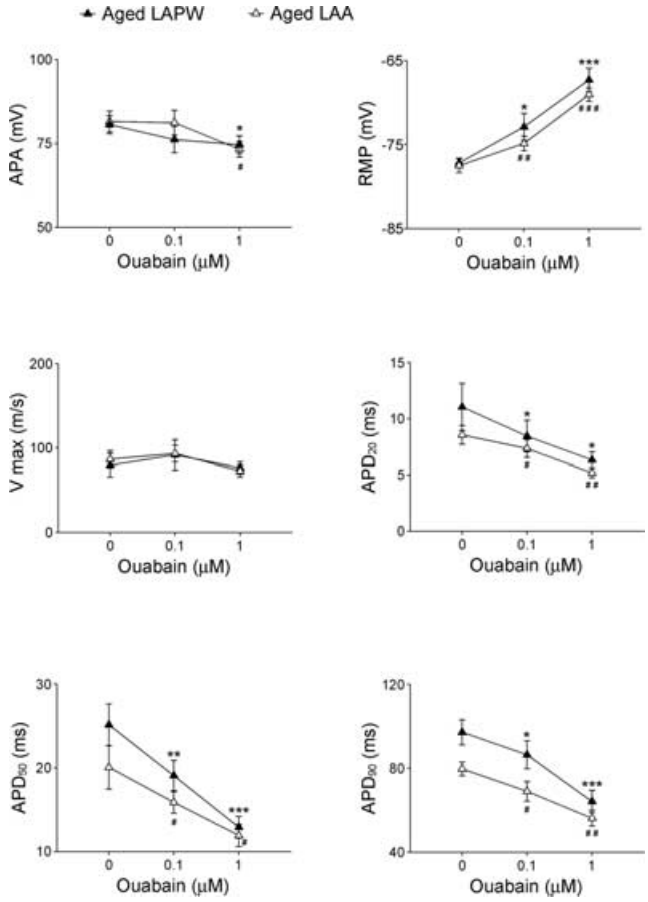

Figure 5. *The average data of the APA, RMP, V_{max}, APD_{20}, APD_{50}, and APD_{90} in the aged LAPWs and LAAs after ouabain (0.1, 1 μM) administration measured during a 2-Hz electrical stimulus. $^{*}P < 0.05$, and $^{**}P < 0.01$, $^{***}P < 0.001$, comparisons between before and after ouabain (0.1, 1 μM) administration in LAPWs. $^{\#}P < 0.05$, and $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$, comparisons between, before, and after ouabain (0.1, 1 μM) administration in LAAs.*

Ouabain Effects on the Heterogeneity of the Aged LA

In the aged LA (Figs. 4 and 5), ouabain (0.1 μM) significantly decreased the RMP and shortened the APD_{20}, APD_{50}, and APD_{90} in both the LAPW (n = 9) and LAA (n = 9). Moreover, ouabain (0.1 μM) induced DADs in 67% of the LAPWs and in 44% of the LAAs ($P > 0.05$). The amplitude of the ouabain-induced DADs (3.1 ± 0.5 mV vs 2.6 ± 0.3 mV, $P > 0.05$) was similar for the LAPW and LAA.

Ouabain (1 μM) significantly decreased the APA and RMP, and shortened the APD_{20}, APD_{50}, and APD_{90}, in both the LAPW and LAA, but did not change the V_{max}. Ouabain (1 μM) induced the occurrence of DADs in 89% of the LAPWs (n = 9) and in 56% of the LAAs (n = 9), respectively, and exhibited a significantly larger amplitude of the DADs (6.7 ± 1.0 mV vs 3.3 ± 0.5 mV, $P < 0.05$) in the LAPW than in the LAA. Moreover, ouabain (1 μM) induced spontaneous activity (Fig. 4B) in 33% of the LAPWs with an average duration of 22.2 ± 18.5 minutes, but did not induce any spontaneous activity in the LAA.

Comparisons Between the Effects of Ouabain in the Young and Aged LAs

Figure 6 shows the changes in the AP parameters after the administration of ouabain (1 μM) in young and aged LAAs and LAPWs. Ouabain (1 μM) shortened the APD_{90} to a greater extent in the aged LAPWs than in the young LAPWs. In the presence of ouabain (1 μM), the aged LAPW tended to have a larger amplitude of the DADs than did the young LAPW ($P = 0.07$).

Discussion

The left atria play an important role in the pathophysiology of AF.[2,3] The results of this study showed that young LAPWs had larger APAs than the young LAAs and that the aged LAPWs had a longer APD_{90} and APD_{50} than the aged LAAs. Those findings proved our hypothesis that regional differences exist in the LA electrophysiological characteristics, and also correlated with the clinical observations that LAPW had a higher incidence of ectopic beat-initiating AF[7] and contributes to the AF maintenance.[16,17] Moreover, the higher DAD incidence in the aged LAPWs suggests that LAPW and aging have a vital role in the AF genesis. The longer APD observed in the aged LAPW might be considered to prevent AF. However, this effect also resulted in an increase in the APD dispersion in the aged LA, which would favor the genesis of reentrant arrhythmias and potentiate the occurrence of AF.

The result of this study showed that aging depolarized the RMP in LAs. This finding agrees with that reported by Anyukhovsky et al.[10] Based on those findings, we suggest that the greater depolarized RMP may imply a greater tendency to cause DADs in triggering arrhythmias and that DADs were only found in the aged rather than young LAs. Since

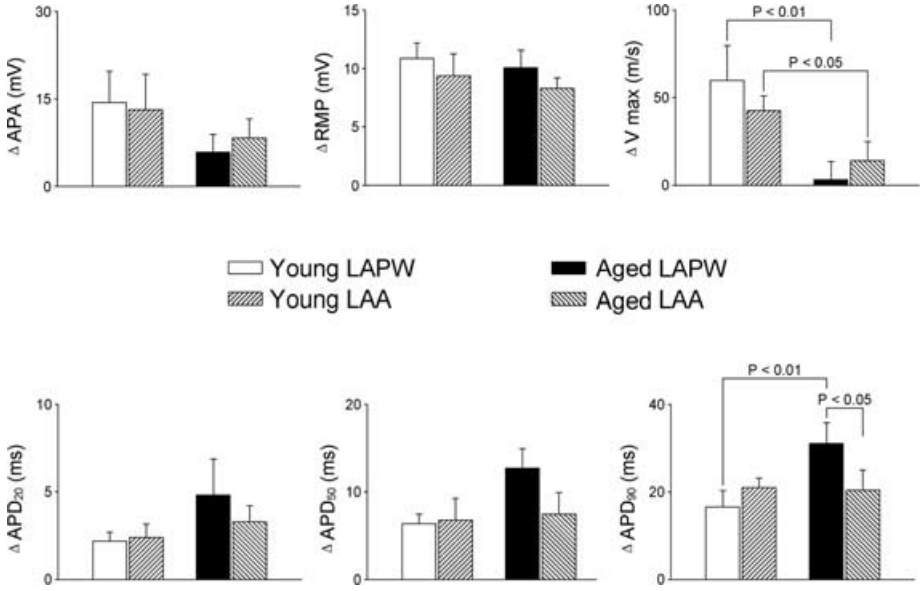

Figure 6. *Changes in the AP parameters after ouabain (1 μM) administration in the young and aged LAAs and LAPWs measured during a 2-Hz electrical stimulus.*

triggered activity is thought to be one of the AF mechanisms, the more aging-related depolarized RMP is, in part, prone to having the higher AF propensity. In addition, smaller APA and V_{max} in the aged LAs might be due to aging-related changes in the Na^+ current kinetics[18] and density.[12] We also found that the aging-associated electrophysiological changes were significantly different between the LAPW and LAA. Aging may have heterogeneous effects on the LA electrical activity. Previous studies have indicated that chronic AF patients have greater histopathologic changes in the LAPW than in LAA.[19,20] The investigators proposed that the regional heterogeneity in the LA may be caused by a greater wall stress in the LAPW due to its thinner wall and larger volume,[19] or attributable to the different embryological origins,[21] as the LAA is developed from the embryonic LA and the LAPW is originated from an outgrowth of the pulmonary veins. Those notions may contribute to the regional difference of electrophysiologic findings in LA as observed in the present study.

In this study, ouabain was found to induce larger amplitudes of the DADs in the LAPW than in the LAA. This result may be explained by the fact that the larger APA in the young LAPWs was supposedly due to greater Na^+ currents. Therefore, inhibiting Na^+/K^+-ATPase by ouabain may increase intracellular Na^+ and induce a greater Ca^{2+} overload to cause DADs through forward Na^+-Ca^{2+} exchanges. Moreover, in the aged LAPW, due to the more calcium influx, the longer APD at baseline may have resulted in larger DADs after administrating ouabain.

Limitations of the Study

The data should be interpreted with caution due to the limitations of this study. First, we investigated the aging effects on the electrophysiological properties only in the LA. Therefore, we are not sure that RA can produce similar aging effects. But, the more important role of LA (rather RA) in AF pathophysiology suggests that our findings provided important insight to understand the aging-related AF vulnerability. Second, the regional heterogeneity was studied only from LAPW and LAA. Although the regional differences between the two sites were clearly shown in the aged LAs, the effects of aging on the other regions in LA were not clear.

Conclusions

Heterogeneity of the LA and distinctive LAPW electrical characteristics were observed. Aging changed the atrial electrophysiology and further enhanced the LAPW arrhythmogenesis.

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References

1. Feinberg WM, Blackshear JL, Laupacis A, Kronmal R, Hart RG: Prevalence, age distribution, and gender of patients with atrial fibrillation. Analysis and implications. Arch Intern Med 1995;155:469-473.
2. Oral H, Scharf C, Chugh A, Hall B, Cheung P, Good E, Veerareddy S, Pelosi F Jr, Morady F: Catheter ablation for paroxysmal atrial fibrillation: Segmental pulmonary vein ostial ablation versus left atrial ablation. Circulation 2003;108:2355-2360.
3. Pappone C, Oreto G, Rosanio S, Vicedomini G, Tocchi M, Gugliotta F, Salvati A, Dicandia C, Calabro MP, Mazzone P, Ficarra E, Di Gioia C, Gulletta S, Nardi S, Santinelli V, Benussi S, Alfieri O: Atrial electroanatomic remodeling after circumferential radiofrequency pulmonary vein ablation: Efficacy of an anatomic approach in a large cohort of patients with atrial fibrillation. Circulation 2001;104:2539-2544.
4. Sueda T, Nagata H, Shikata H, Orihashi K, Morita S, Sueshiro M, Okada K, Matsuura Y: Simple left atrial procedure for chronic atrial fibrillation associated with mitral valve disease. Ann Thorac Surg 1996;62:1796-1800.
5. Wu TJ, Doshi RN, Huang HL, Blanche C, Kass RM, Trento A, Cheng W, Karagueuzian HS, Peter CT, Chen PS: Simultaneous biatrial computerized mapping during permanent atrial fibrillation in patients with organic heart disease. J Cardiovasc Electrophysiol 2002;13:571-577.
6. Huang JL, Tai CT, Lin YJ, Ting CT, Chen YT, Chang MS, Lin FY, Lai WT, Chen SA: The mechanisms of an increased dominant frequency in the left atrial posterior wall during atrial fibrillation in acute atrial dilatation. J Cardiovasc Electrophysiol 2006;17:178-188.
7. Chen SA, Hsieh MH, Tai CT, Tsai CF, Prakash VS, Yu WC, Hsu TL, Ding YA, Chang MS: Initiation of atrial fibrillation by ectopic beats originating from the pulmonary veins: Electrophysiological characteristics, pharmacological responses, and effects of radiofrequency ablation. Circulation 1999;100:1879-1886.
8. Feng J, Yue L, Wang Z, Nattel S: Ionic mechanisms of regional action potential heterogeneity in the canine right atrium. Circ Res 1998;83:541-551.

9. Anyukhovsky EP, Sosunov EA, Plotnikov A, Gainullin RZ, Jhang JS, Marboe CC, Rosen MR: Cellular electrophysiologic properties of old canine atria provide a substrate for arrhythmogenesis. Cardiovasc Res 2002;54:462-469.
10. Anyukhovsky EP, Sosunov EA, Chandra P, Rosen TS, Boyden PA, Danilo P Jr, Rosen MR: Age-associated changes in electrophysiologic remodeling: A potential contributor to initiation of atrial fibrillation. Cardiovasc Res 2005;66:353-363.
11. Spach MS, Dolber PC: Relating extracellular potentials and their derivatives to anisotropic propagation at a microscopic level in human cardiac muscle. Evidence for electrical uncoupling of side-to-side fiber connections with increasing age. Circ Res 1986;58:356-371.
12. Wu CC, Su MJ, Chi JF, Wu MH, Lee YT: Comparison of aging and hypercholesterolemic effects on the sodium inward currents in cardiac myocytes. Life Sci 1997;61:1539-1551.
13. Sticherling C, Oral H, Horrocks J, Chough SP, Baker RL, Kim MH, Wasmer K, Pelosi F, Knight BP, Michaud GF, Strickberger SA, Morady F: Effects of digoxin on acute, atrial fibrillation-induced changes in atrial refractoriness. Circulation 2000;102:2503-2508.
14. Tieleman RG, Blaauw Y, Van Gelder IC, De Langen CD, de Kam PJ, Grandjean JG, Patberg KW, Bel KJ, Allessie MA, Crijns HJ: Digoxin delays recovery from tachycardia-induced electrical remodeling of the atria. Circulation 1999;100:1836-1842.
15. Wongcharoen W, Chen YC, Chen YJ, Chang CM, Yeh HI, Lin CI, Chen SA: Effects of a Na^+/Ca^{2+} exchanger inhibitor on pulmonary vein electrical activity and ouabain-induced arrhythmogenicity. Cardiovasc Res 2006;70:497-508.
16. Kalifa J, Tanaka K, Zaitsev AV, Warren M, Vaidyanathan R, Auerbach D, Pandit S, Vikstrom KL, Ploutz-Snyder R, Talkachou A, Atienza F, Guiraudon G, Jalife J, Berenfeld O: Mechanisms of wave fractionation at boundaries of high-frequency excitation in the posterior left atrium of the isolated sheep heart during atrial fibrillation. Circulation 2006;113:626-633.
17. Sanders P, Berenfeld O, Hocini M, Jais P, Vaidyanathan R, Hsu LF, Garrigue S, Takahashi Y, Rotter M, Sacher F, Scavee C, Ploutz-Snyder R, Jalife J, Haissaguerre M: Spectral analysis identifies sites of high-frequency activity maintaining atrial fibrillation in humans. Circulation 2005;112:789-797.
18. Baba S, Dun W, Hirose M, Boyden PA: Sodium current function in adult and aged canine atrial cells. Am J Physiol 2006;291:H756-H761.
19. Corradi D, Callegari S, Benussi S, Maestri R, Pastori P, Nascimbene S, Bosio S, Dorigo E, Grassani C, Rusconi R, Vettori MV, Alinovi R, Astorri E, Pappone C, Alfieri O: Myocyte changes and their left atrial distribution in patients with chronic atrial fibrillation related to mitral valve disease. Hum Pathol 2005;36:1080-1089.
20. Corradi D, Callegari S, Benussi S, Nascimbene S, Pastori P, Calvi S, Maestri R, Astorri E, Pappone C, Alfieri O: Regional left atrial interstitial remodeling in patients with chronic atrial fibrillation undergoing mitral-valve surgery. Virchows Arch 2004;445:498-505.
21. Sadler TW: Cardiovascular system. In: Langman J, ed. *Langman's Medical Embryology*. Baltimore: Williams and Wilkins, 1990, pp. 179-227.