Gender Differences in the Electrophysiological Characteristics of Pulmonary Veins and Left Atrium and Their Clinical Implication in Atrial Fibrillation

Running title: Tsai et al.; Gender differences in AF

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Abstract:

*Background* - Gender and the autonomic nervous system play critical roles in the pathophysiology of atrial fibrillation (AF). Gender differences in electrophysiological characteristics of the pulmonary veins (PVs, AF initiator) and left atrium (LA, AF substrate) are not clear.

*Methods and Results* - Conventional microelectrodes were used to record the action potential (AP) in isolated PV and LA tissue preparations from male and female (aged 8–10 months) rabbits before and after drug administration (adenosine, acetylcholine and isoproterenol). Male PVs ($n=7$) had a higher spontaneous beating rate (1.7±0.2 vs. 1.2±0.1 Hz, $p=0.021$) and incidence of burst firing (72% vs. 11%, $p=0.038$) than female PVs ($n=9$). Male PVs without spontaneous activity ($n=10$) and the LA ($n=11$) had longer AP durations (APDs) than female PVs ($n=9$) and LA ($n=9$). Additionally, male PVs had a more-positive resting membrane potential (79±3 vs. 84±2 mV, $p=0.022$). Isoproterenol (3 μM) increased the delayed afterdepolarizations (DADs) to a greater extent in male than in female PVs. In PVs without spontaneous activity or LA, isoproterenol (0.1 and 3 μM) consistently shortened the APDs in females but not in males. Acetylcholine (5.5 μM) decreased the spontaneous activity of PVs and shortened the APDs in both groups. Adenosine (10 μM) also similarly decreased the spontaneous activity of PVs and DADs in both groups.

*Conclusions* - There are significant gender differences in PV and LA AP characteristics in rabbits. The higher amplitude of DADs after isoproterenol superfusion in male PVs may contribute to gender-related arrhythmogenesis.

**Key words:** Atrial fibrillation; Gender; Pulmonary veins; Left atrium.
Introduction

Atrial fibrillation (AF) is the most common cardiac arrhythmia seen in clinical practice and induces cardiac dysfunction and stroke.\(^1\)\(^,\)\(^2\) Gender differences were shown to play important roles in the pathogenesis of AF.\(^3\) In the Framingham study, the prevalence of AF was significantly greater in men than in women. In addition, a large gender difference was found in a more recent survey.\(^4\) However, the mechanisms underlying the lower incidence of AF in females are not clear.

Pulmonary veins (PVs) are important AF initiators and the left atrium (LA) is the main AF substrate for reentry.\(^5\)\(^,\)\(^6\) PVs contain a mixture of pacemaker cells and working myocardium, and were suggested to be a subsidiary pacemaker that can induce atrial arrhythmias.\(^7\)\(^-\)\(^10\) Also, animal and human studies showed that activity during AF is more rapid in the LA than in the right atrium.\(^11\)\(^-\)\(^13\) Those studies suggested that high-frequency sources in the LA act as triggers and/or drivers for some types of AF. A previous study indicated a different probability of PV- and non-PV-related paroxysmal AFs between males and females, which suggested that gender differences may have electrophysiological effects that result in different AF incidences.\(^14\)\(^,\)\(^15\) Female ventricular myocytes have longer action potential (AP) durations (APDs) caused by differences in \(K^+\) currents and L-type calcium currents.\(^16\)\(^-\)\(^19\) However, gender differences in the LA and PV electrophysiology have not been elucidated.

The autonomic nervous system plays a critical role in the pathophysiology of AF.\(^20\)\(^-\)\(^22\) Isoproterenol was shown to enhance PVs by increasing automaticity and triggering activity.\(^23\)\(^,\)\(^24\) Acetylcholine-mediated premature atrial beats can also trigger reentrant excitation and AF.\(^25\)\(^,\)\(^26\) Moreover, adenosine can induce APD-shortening, premature atrial beats or AF.\(^27\)\(^-\)\(^29\) Adenosine can also transiently restore conduction through a previously isolated PV, which can reduce AF recurrence after pulmonary veins isolation.\(^30\)\(^,\)\(^31\) In addition, adenosine
increased atrial dominant frequency, and mathematical modeling suggested an effect to increase reentrant drivers.\textsuperscript{32} Since gender differences are associated with different autonomic nervous activities, it is possible that there are gender-related differences in the responses to autonomic nervous agents, resulting in different AF genesis. Therefore, the purposes of this study were to evaluate gender differences in the electrophysiology of PVs and the LA, and investigate the effects of autonomic agents and adenosine on different genders.

**Materials and Methods**

**Rabbit PVs and LA appendage preparations**

This investigation conformed to the institutional Guide for the Care and Use of Laboratory Animals. Male and female rabbit (aged 8~10 months and weighing 3.0~4.0 kg) were anesthetized with an intraperitoneal injection of sodium pentobarbital (40 mg/kg). A midline thoracotomy was performed, and the heart and lungs were removed. For dissection of the PVs and LA, the LA was opened by an incision along the mitral valve annulus extending from the coronary sinus to the septum in Tyrode's solution with a composition (in mM) of 137 NaCl, 4 KCl, 15 NaHCO\(_3\), 0.5 NaH\(_2\)PO\(_4\), 0.5 MgCl\(_2\), 2.7 CaCl\(_2\), and 11 dextrose. The PVs were separated from the atrium at the LA-PV junction and separated from the lungs at the ending of the PV myocardial sleeves. The prepared veins were about 10 mm long and 5 mm wide. One end of the preparation, consisting of the PVs, LA-PV junction, and atrial tissue (within 1 mm in length), was pinned with needles to the bottom of a tissue bath. The other end (the distal PV) was connected to a Grass FT03C force transducer with silk thread. The adventitia or epicardial side of the preparations faced upwards. Tissue strips (~10 × 5 × 0.5 mm) from the LA were then dissected, and prepared the same as were the PVs. The tissue was superfused at a constant rate of 3 ml/min with Tyrode's solution which was saturated
with a 97% O\textsubscript{2} and 3% CO\textsubscript{2} gas mixture. The temperature remained constant at 37 °C, and the preparations were allowed to equilibrate for 1 h before the electrophysiological study.

**Electrophysiological and pharmacological studies**

Transmembrane APs of the PVs and LA were recorded by means of machine-pulled glass capillary microelectrodes filled with 3 M KCl, and tissue preparations were connected to a WPI model FD223 electrometer under a tension of 150 mg. Thus, the proarrhythmia seen at the baseline was stretch-induced. Electrical and mechanical events were simultaneously displayed on a Gould 4072 oscilloscope and Gould TA11 recorder. Signals were recorded with DC coupling and a 10-KHz low-pass filter cutoff frequency using a data acquisition system. The electrical stimulus was provided by a Grass S88 stimulator through a Grass SIU5B stimulus isolation unit. Spontaneous activity was defined as a constant occurrence of spontaneous APs using no electrical stimuli. Burst firing was defined as accelerated spontaneous activity which was 2-times faster than the baseline beating activity with the characteristics of sudden onset and termination. Early afterdepolarizations (EADs) were defined as interruption of the smooth contour of phase 2 or 3 of the APs. Delayed afterdepolarizations (DADs) were defined as the presence of a spontaneous hump-shaped depolarization of the impulse after full repolarization had occurred. The EADs and DADs were selected from consistent deflections without abrupt changes of resting membrane potential and action potential morphology. APs were elicited through a 2-Hz electrical stimulus before and after drug administration. The resting membrane potential (RMP) was measured during the period between the last repolarization and onset of the subsequent AP. The AP amplitude (APA) was obtained from the RMP to the peak of the AP depolarization. The APDs at repolarization extents of 90%, 50%, and 20% of the APA were measured and respectively designated APD\textsubscript{90}, APD\textsubscript{50}, and APD\textsubscript{20}. Similar to the previous studies
different concentrations of adenosine (0 and 10 μM), acetylcholine (0 and 5.5 μM), and isoproterenol (0, 0.1, and 3 μM) were superfused for at least 10 min to test the pharmacologic effects on the tissue preparations. To avoid contamination with previously used drugs, APs were compared between the baseline and after the washout period for each drug. The effects of all 3 drugs used in this study were completely reversed after being washed out.

**Statistical methods**

All continuous variables are expressed as the mean±S.E.M. Baseline male and female electrophysiological characteristics were compared by Mann-Whitney rank sum test or unpaired t test, depending on the outcome of normality test. Two-way repeated measures analysis of variance (ANOVA) followed by the Bonferroni analysis was used to compare the differences before and after drug administration, and interactions of gender and dose. Nominal variables were compared by a Chi-squared analysis with Yates correction or Fisher’s exact test. p<0.05 was considered significant.

**Results**

**Electrophysiological characteristics of male and female PVs and LA**

Seven of 11 (63%) male rabbits and 9 of 14 (64%) female rabbits (p=0.999) were found to have spontaneous PV activity. Female PVs had significantly slower beating rates (1.2±0.1 vs. 1.7±0.2 Hz, p=0.021) than male PVs (Figure 1A). As the example shown in Figure 1A, male PVs with spontaneous activity had a significantly higher incidence (72%, 4.0±0.4 Hz vs. 11%, 2.1 Hz p=0.038) of burst firing than female PVs with spontaneous activity. However, DADs of male and female PVs had similar incidences (42% vs. 34%, p=0.71) and amplitudes (1.6±0.2 vs. 1.5±0.3 mV, p=0.73) (Figure 1B).
In PVs without spontaneous activity, male PVs had a significantly greater depolarized RMP and a longer APD$_{90}$ than those of female PVs. The male LA also had significantly longer APD$_{90}$, APD$_{50}$, and APD$_{20}$ than the female LA. However, the male and female LA had similar APAs and RMPs (Table, Figure 1C).

**Effects of isoproterenol on male and female PVs and LA**

In PVs with spontaneous activity, isoproterenol (0.1 and 3 μM) concentration-dependently increased the spontaneous rates in male and female PVs (Figure 2A). Isoproterenol (0.1 and 3 μM) induced the occurrence of sustained accelerated spontaneous PV activity to a similar extent between male (72% and 100%) and female PVs (67% and 89%, $p=0.65$, Figure 2B). Isoproterenol (0.1 and 3 μM) had a tendency to increase the incidence of DADs from 33% to 67% and 89% in male PVs ($n=9$, $p=0.053$) and from 33% to 50% and 75% in female PVs ($n=12$, $p=0.101$). However, isoproterenol at a concentration of 3 μM exhibited a significantly higher amplitude of DADs in male PVs than in female PVs (4.3±0.3 vs. 2.2±0.1 mV, $p<0.001$, Figure 2C). After washing out the isoproterenol, the PV automatic activity completely returned to the frequency before drug administration.

In female PVs without spontaneous activity, isoproterenol (0.1 and 3 μM) consistently increased the APA; and isoproterenol (3 μM) also depolarized the RMP and shortened the APD$_{90}$ (Figure 3). In contrast, isoproterenol (0.1 and 3 μM) shortened the APD$_{90}$ in 5 (56%) of 9 male PVs, but prolonged the APD$_{90}$ in the other 4 male PVs (Figure 3). The average data show that isoproterenol (3 μM) hyperpolarized the RMP and increased the APA in male PVs.

In the female LA, isoproterenol (0.1 and 3 μM) consistently increased the APA and shortened APD$_{90}$; and isoproterenol (3 μM) also depolarized the RMP (Figure 3). Similar to that in PVs without spontaneous activity, isoproterenol (0.1 and 3 μM) shortened the APD$_{90}$ in 5 (50%) of 10 male LAs, but prolonged the APD$_{90}$ in the other 4 male LAs (Figure 3).
average data showed that isoproterenol (0.1 and 3 μM) hyperpolarized the RMP and increased the APA in the male LA.

Effects of adenosine on male and female PVs and LA

In PVs with spontaneous activity, adenosine (10 μM) significantly decreased the spontaneous rates in male and female PVs (Figure 4A). Adenosine (10 μM) had a tendency to decrease the incidence of burst firing in male (n=7) PVs (72% vs. 14 %, p=0.105), but not in female (n=9) PVs (11% vs. 11%). Adenosine (10 μM) suppressed the burst firing rates in male PVs from 4.0±0.4 to 0.2±0.2 Hz (p<0.001) and in one female PV from 2.1 to 1.8 Hz (Figure 4B). Adenosine (10 μM) decreased the amplitude of DADs in male PVs (from 1.6±0.2 to 1.0±0.1 mV, n=3, p=0.001) and in female PVs (from 1.5±0.1 to 0.8±0.1 mV, n=4, p=0.001, Figure 4C) to a similar extent. After adenosine had been washed out, the PV automatic activity completely returned to the frequency before drug administration.

In PVs without spontaneous activity, adenosine (10 μM) significantly hyperpolarized the RMP and increased the APA in female PVs but not in male PVs (Figure 5). Compared to the male LA, adenosine (10 μM) shortened the APD₉₀ and APD₅₀ to greater extents in the female LA (Figure 5).

Effects of acetylcholine on male and female PVs and LA

In PVs with spontaneous activity, acetylcholine (5.5 μM) significantly decreased the spontaneous rates in male and female PVs (Figure 6A). Acetylcholine (5.5 μM) had a tendency to decrease the incidence of burst firing in male (n=7) PVs (72% vs. 14 %, p=0.105), but not in female (n=9) PVs (11% vs. 11%). Acetylcholine (5.5 μM) suppressed the burst firing rates in male PVs from 4.0±0.5 to 2.3±0.5 Hz (p=0.007) and in one female PV from 2.1 to 1.7 Hz. In addition, acetylcholine (5.5 μM) completely suppressed PV spontaneous activity.
in 50% of male PVs and in 72% of female PVs (Figure 6B); and suppressed the incidences of DADs in male PVs (from 36% to 0%) and in female PVs (from 36% to 7%) to a similar extent (Figure 6C). The spontaneous activity was not reversible after washing out the acetylcholine.

In PVs without spontaneous activity, acetylcholine (5.5 μM) significantly hyperpolarized the RMP, and shortened the APD_{90}, APD_{50}, and APD_{20} in both male and female PVs and LA to similar extents (Figure 7), which were different from the effects of adenosine on male LA. However, acetylcholine (5.5 μM) significantly increased the APA in the female LA, but not in the male LA (Figure 7).

**Discussion**

Gender has significant associations with cardiac electrophysiology and the genesis of AF. In this study, for the first time, we demonstrated that there are gender-related differences in PV and LA electrical characteristics. The faster spontaneous activity, and higher incidence of burst firing, and less-negative RMP in male PVs may facilitate the genesis of triggered activity and automaticity. These findings in rabbits implied that higher male PV arrhythmogenesis may contribute at least partially to the higher incidence of AF in males. Moreover, it was shown that women have higher sinoatrial node activity before and after autonomic blockade. In the present study, we demonstrated that male PVs had a faster spontaneous rate than female PVs. Since PVs have to compete with the sinoatrial node to induce atrial arrhythmia, the slower PV beating rates and faster sinoatrial node activity in females may
contribute to lower arrhythmogenesis from the PV and AF. To the best of our knowledge, there were limited data related to the electrophysiological difference of PVs and LA among the gender. In female, there are dynamic changes in QT intervals and torsade de pointes risks during the menstrual cycle and pregnancy, which may be related with serum ovarian steroids. Therefore, menstrual phase-dependent differences of PV and LA electrophysiology in female animals may provide a clue to elucidate molecular mechanisms.

In this study, similar to previous observations, isoproterenol triggered and increased the male and female spontaneous PV activity, which suggests the importance of β adrenergic stimulation in PV arrhythmogenic activity. Compared to female PVs, isoproterenol (3 μM) increased the amplitude of DADs to a greater extent in male PVs. These findings may result in higher PV arrhythmia during enhanced sympathetic activity. In addition, in contrast to the consistent shortening of APDs by isoproterenol in female PVs and LA, isoproterenol might prolong or shorten the APDs in male PVs and LA. However, such inconsistency of adrenergic modification of APDs among male PVs and LA cannot directly account for vulnerability to reentry. Experimental data of spatial dispersion and/or temporal alteration of APDs or effective refractory period may be required. Moreover, previous studies have found important species and chamber differences in the APDs responses to isoproterenol. In humans, isoproterenol (50 nM) induces the genesis DADs and prolongs the APDs in human atrial myocytes. Since adrenergic stimulation is important in the genesis of AF, the
greater amplitude of isoproterenol-induced DADs in male rabbit PVs may significant contribute to gender differences on arrhythmogenesis.

It is well known that vagal nerve stimulation and an acetylcholine infusion can result in significant changes to the cardiac electrophysiology. In the current study, similar to a previous study on canine PVs, acetylcholine decreased the spontaneous activity and burst firing in both male and female PVs. However, acetylcholine shortened the APDs of PVs and LA in males and females to similar extents, which may facilitate induction of AF.

In this study, we found that adenosine decreased the spontaneous activity in both male and female PVs. These effects may be caused by the known antiadrenergic effect of adenosine by cAMP-dependent inhibition of $I_{Ca,L}$. However, adenosine only decreased the male PV burst firing, but not that of female PVs. The possible mechanism may be the higher catecholamine status in males.

Adenosine and acetylcholine are known to activate the same Kir3.x subfamily of inward rectifier potassium channels through different signaling pathways. By increasing $K^+$ conductance in the atrium, both acetylcholine and adenosine hyperpolarize the cell membrane, abbreviate the APDs, and inhibit spontaneous pacemaker discharge, as well as EADs and DADs. In our study, both adenosine and acetylcholine had similar response to RMP, APDs, DADs but adenosine could shorten the APDs in the female LA but not in the male LA. A previous study pointed out that females expressed higher levels of the functional cardiac $K^+$-
ATP channel than men, which implies a different composition of the K^+ channel between genders.\textsuperscript{48}

**Conclusion**

There are significant gender differences in PV and LA AP characteristics in rabbits. The higher amplitude of isoproterenol-induced DADs in male PVs may contribute to gender-related arrhythmogenesis.

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**Conflict of Interest Disclosures:** None

**References:**


Table. Baseline electrophysiologic characteristics of pulmonary veins (PVs) and left atrium (LA) in male and female rabbits

<table>
<thead>
<tr>
<th>Electrophysiologic property</th>
<th>PVs</th>
<th>LA</th>
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<tbody>
<tr>
<td></td>
<td>Male (n=10)</td>
<td>Female (n=9)</td>
</tr>
<tr>
<td>RMP (-mV)</td>
<td>78.9±2.6</td>
<td>83.9±2.2*</td>
</tr>
<tr>
<td>APA (mV)</td>
<td>104.9±1.2</td>
<td>100.3±2.5</td>
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<tr>
<td>APD$_{90}$ (ms)</td>
<td>104.2±5.9</td>
<td>86.6±4.6*</td>
</tr>
<tr>
<td>APD$_{50}$ (ms)</td>
<td>36.7±4.9</td>
<td>33.2±4.5</td>
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<tr>
<td>APD$_{20}$ (ms)</td>
<td>8.4±1.5</td>
<td>7.7±1.2</td>
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RMP, resting membrane potential; APA, action potential amplitude; APD$_{20}$, APD$_{50}$, APD$_{90}$, 20, 50, 90 % of the action potential duration. * $p<0.05$, vs. respective males PVs or LA

Figure Legends:

Figure 1. Action potential (AP) from male and female pulmonary veins (PVs) and left atrium (LA). (A). Faster spontaneous rates (upper panel) and burst firing (lower panel) were found in male PVs than in female PVs. (B). Delayed afterdepolarizations occur in male or female PVs. (C) Tracings revealed longer AP durations in male PVs and LA than in female PVs and LA with a 2-Hz electrical stimulus.

Figure 2. Effects of isoproterenol on male and female pulmonary veins (PVs) with spontaneous activity. (A). Tracings and concentration–response curve of increased spontaneous rates in both male (n=7) and female (n=9) PVs after isoproterenol (0.1 and 3 μM). (B). Tracings revealed sustained firing (upper panel) and early afterdepolarizations (lower panel) occurrences after isoproterenol (3 μM) in male and female PVs. (C). Examples of isoproterenol (0.1 and 3 μM)-induced increased amplitude of delayed afterdepolarizations.
(DADs) in both male and female PVs. There was a higher amplitude of DADs in male PVs than in female PVs after isoproterenol treatment (3 µM). *p<0.05 vs baseline in male PVs. # p<0.05 vs baseline in female PVs.

Figure 3. Effects of isoproterenol on male and female left atrium (LA) and pulmonary veins (PVs) without spontaneous activity. Superimposed tracings show the effects of different concentrations of isoproterenol on action potential (AP) configurations. The upper panel shows the progressive lengthening of the AP duration caused by isoproterenol in male PVs and LA and the middle panel shows the progressive shortening of the AP duration caused by isoproterenol in male PVs and LA. The lower panel showed the progressive shortening of the AP duration caused by isoproterenol in female PV and LA. Changes in the AP parameters after isoproterenol (0.1 and 3 µM) administration in the male and female PV and LA measured during a 2-Hz electrical stimulus. * p<0.05, ** p<0.01, *** p<0.005.

Figure 4. Effects of adenosine on male and female pulmonary veins (PVs) with spontaneous activity. (A). Tracings and average data of adenosine (10 µM) on male (n=7) and female (n=9) PVs with spontaneous activity. (B). Adenosine (10 µM) decreased the frequency of burst firing in male and female PVs. (C). Examples of decreased amplitude of delayed afterdepolarizations in both male and female PVs after adenosine (10 µM) administration. *p<0.05 vs before adenosine administration in male PVs. # p<0.05 vs before adenosine administration in female PVs.

Figure 5. Effects of adenosine on male and female left atrium (LA) and pulmonary veins (PVs) without spontaneous activity. Superimposed tracings of the effects of adenosine on action potential (AP) configurations. Changes in the AP parameters after adenosine (10 µM)
administration in the male and female PVs and LA measured during a 2-Hz electrical stimulus. * p<0.05, ** p<0.01, *** p<0.005.

Figure 6. Effects of acetylcholine on male and female pulmonary veins (PVs) with spontaneous activity. (A). Tracings and average data of acetylcholine (5.5 μM) on male (n=7) and female (n=9) PVs with spontaneous activity. (B). Acetylcholine (5.5 μM) decreased amplitude of delayed afterdepolarizations in both male and female PVs. (C). Acetylcholine (5.5 μM) induced silent activity in both male and female PVs. *p<0.05 vs before acetylcholine administration in male PVs. # p<0.05 vs before acetylcholine administration in female PVs.

Figure 7. Effects of acetylcholine on male and female left atrium and pulmonary veins (PVs) without spontaneous activity. The superimposed tracings of the action potential (AP) configuration before and after the acetylcholine (5.5 μM) with a 2-Hz electrical stimulus.* p<0.05, ** p<0.01, *** p<0.005.
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