Changes in Atrial Effective Refractory Period and $I_{K_ACh}$ After Vagal Stimulation Plus Rapid Pacing in the Pulmonary Vein

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**Introduction and objectives.** Recent studies have shown that rapid atrial pacing causes atrial electrical remodeling. However, the influence of the vagus nerve on atrial electrical remodeling is not clear.

**Methods.** This study involved 24 dogs divided into 3 groups. In the control group, the inducibility of atrial fibrillation (AF) during vagal stimulation (VS) was investigated. In the pacing group, the atrial effective refractory period (AERP) was determined before and after pacing in the left superior pulmonary vein (LSPV). In the vagal stimulation (VS) plus pacing group, the LSPV was subjected to rapid electrical pacing after vagal stimulation (VS), and the AERP was measured both before VS and after pacing. The $I_{K_ACh}$ density was measured in LSPV and atrial myocardial cells in the 3 groups using the patchclamp technique.

**Results.** The duration of induced AF was greater in the pacing group than in the control or VS-plus-pacing group. In the pacing group, the AERP was markedly shortened and the AERP dispersion (dAERP) was significantly decreased ($P<0.05$). However, there was no significant change in AERP in the VS-plus-pacing group, though the dAERP increased significantly ($P<0.05$). The $I_{K_ACh}$ density was increased in LSPV and atrial myocardial cells after pacing. However, there was no significant change in $I_{K_ACh}$ density after VS plus pacing.

**Conclusions.** Although shortening of the AERP may play a fundamental role, it is not in itself responsible for cholinergically induced AF. Rapid pacing in the LSPV increased the $I_{K_ACh}$. However, VS before rapid pacing partly protected the atria against electrical remodeling.

**Key words:** Vagus nerve. Electrical remodeling. Atrial fibrillation. PACing. Dogs.

**Introducción y objetivos.** Estudios recientes han demostrado que la estimulación auricular rápida causa un remodelado eléctrico auricular. Sin embargo, no se ha aclarado el efecto del nervio vago en el remodelado eléctrico auricular.

**Métodos.** Se dividió un total de 24 perros en 3 grupos. En el grupo control, se estudió la inducibilidad de fibrilación auricular (FA) durante la estimulación vagal (EV). En el grupo de estimulación eléctrica (EE), se determinó el periodo refractario efectivo auricular (PREA) antes y después de la estimulación en la vena pulmonar superior izquierda (VPSI). En el grupo de EV + EE, tras la estimulación vagal (EV$_2$), se aplicó EE rápida en la VPSI. Se determinó el PREA antes de la EV$_2$ y después de la EE. Se determinaron las densidades de $I_{K_ACh}$ en las células de VPSI y de miocardio auricular mediante la técnica de patch clamp en los 3 grupos.

**Resultados.** La duración de la FA inducida en el grupo de EE fue superior a la de los grupos de control y de EV + EE. En el grupo de EE, el PREA presentó un acortamiento marcado y la dispersión del PREA (dPREA) aumentó de forma significativa ($p < 0.05$). Sin embargo, en el grupo de EV+EE el PREA no se modificó de forma significativa, mientras que la dPREA aumentó significativamente ($p < 0.05$). Las densidades de $I_{K_ACh}$ aumentaron en las células de VPSI y del miocardio auricular tras la aplicación de la estimulación eléctrica. Sin embargo, no hubo diferencias significativas en las densidades de $I_{K_ACh}$ tras la EV$_2$ más EE.

**Conclusiones.** La disminución del PREA puede ser fundamental, pero no es de por sí causa de la inducción colinérgica de la FA. La estimulación rápida en la VPSI aumentó la $I_{K_ACh}$. Sin embargo, la EV antes de la EE rápidamente protege en parte a las aurículas contra el remodelado eléctrico.

**Palabras clave:** Vago. Remodelado eléctrico. Fibrilación auricular. Estimulación eléctrica. Perros.
INTRODUCTION

Recent experimental and clinical studies have established the role of the autonomic nervous system, particularly the parasympathetic nervous system, in the pathogenesis of atrial fibrillation (AF). However, few reports have focused on the relationship between parasympathetic tone and recovery from electrical remodeling. Blaauw et al reported that high vagal tone was associated with a short atrial effective refractory period (AERP) after rapid pacing and that there was a prolonged recovery from remodeling in goats. Miyauchi et al demonstrated that blockage of the parasympathetic system may facilitate early recovery from electrical remodeling associated with short-term rapid pacing. However, in another study, Takei et al showed that VS prior to rapid pacing prevented electrical remodeling. It is widely accepted that VS is mediated by release of acetylcholine-regulated receptors and activates the atrial acetylcholine-regulated potassium current (I\textsubscript{KACH}); consequently there is shortening of the AERP, the atrial action potential duration (APD), and that enhances the dispersion of the AERP (dAERP), inducing AF. The changes in atrial electrical properties (electrical remodeling) are associated with the activation of I\textsubscript{KACH}. Thus, we hypothesize that the effect of vagal tone on atrial electrical remodeling is related to the densities of I\textsubscript{KACH}. To study the mechanism of the effect of vagal tone on electrical remodeling, we investigated the changes in the AERP and I\textsubscript{KACH} after VS and rapid pacing in left superior pulmonary vein (LSPV) and discuss the relationship between parasympathetic tone and recovery after electrical remodeling.

METHODS

Experimental Animals

Twenty-four dogs, weighing 15 to 22 kg (mean, 20 [3] kg) were used in the study. The animals were anesthetized via the abdominal route with pentobarbital sodium (30 mg/kg body weight) and ventilated with room air. After a median sternotomy, the heart was exposed in a pericardial cradle. The bilateral cervical vagal trunks were then severed to impede the arrival of all tonic neural activity to the heart. Continuous electrocardiographic monitoring was carried out using leads II and aVF.

Electrophysiological Measurements

The 3 custom-built electrode probes were applied with an electrode operator (UNM-1, Japan) to the right and left atrial epicardial surfaces, and to the LSPV. Reference electrodes were fixed to the chest wall. The AERP was determined by a LEAD-2000B instrument (Sichuan, China). Electrode probe electrograms were filtered at 30-500 Hz. Electrocardiographic filter settings ranged from 0.1 to 250 Hz. The S\textsubscript{1}-S\textsubscript{2} intervals were decreased from 150 ms to refractoriness, initially by decrements of 10 ms (S\textsubscript{1}-S\textsubscript{2}=8:1). As the S\textsubscript{1}-S\textsubscript{2} intervals approached the AERP, decrements were reduced to 5 ms. An extra stimulus (S\textsubscript{3}) was added late in atrial diastole, and the interval between S\textsubscript{1} and S\textsubscript{2} was reduced in 5-ms steps until there was no propagated atrial response. The longest S\textsubscript{1-S\textsubscript{2}} coupling interval that failed to result in a propagated atrial response was taken as the local AERP.

Experiment Protocol

Twenty-four dogs, divided into 3 groups of 8 each, were used for the study as follows: control group, pacing group and VS-plus-pacing group.

In the control group, VS was achieved by introducing silver wires into the right cranial end of the vagosympathetic trunk towards the canine heart. Electrical stimulation was then delivered at a frequency of 20 Hz, in pulses of 0.2-ms duration (electrophysiological stimulator SEN-7103, Japan). The voltage chosen for VS was 5 V above the voltage at which a sinus arrest lasting over 2 seconds was achieved. This stimulation protocol was referred to as VS\textsubscript{1}. The inducibility of AF was assessed during the same period. When AF was induced, VS\textsubscript{1} was concluded. If after 15 seconds of VS\textsubscript{1}, AF was not induced, electrical stimulation was also discontinued (Figure 1).

In the pacing group, 8 dogs were subjected to LSPV pacing at 500 beats/minute for 4 hours. The AERP was measured in right atrium (RA), left atrium (LA), and LSPV both before and after pacing, after which, VS was recorded and the inducibility of AF was again measured.

In the VS-plus-pacing group, silver wires were introduced into the right vagosympathetic trunks towards the canine hearts. After determining the
AERP, electrical stimulation was delivered at a frequency of 5 Hz, in pulses of 0.2 ms duration and at a voltage of 5-10 V for 30 minutes. This stimulation protocol was referred to as VS<sub>2</sub>. We selected a lower stimulation frequency for VS<sub>2</sub> to avoid second or third-degree atrioventricular block and permitted atrial pacing to be conducted to the ventricle during VS<sub>2</sub>. The LSPV was then subjected to rapid pacing at 500 beats/min for 4 hours. After cessation of pacing, the AERP was measured and AF inducibility was assessed again during VS<sub>1</sub>. The ΔAERP was calculated by determining the difference between the highest and lowest AERP from 3 AERP recorded at the same time.

**Patch-clamp Techniques**

After electrophysiological measurements, the canine hearts were excised and immersed in normal saline at 0°C. The tissues were dissected from the RA, LA, and LSPV were immediately kept in 3 separate beakers containing Ca<sup>2+</sup>-free Tyrode solution (30 mL) containing 136 mM NaCl, 5.4 mM KCl, 1 mM MgCl<sub>2</sub>, 0.33 mM NaH<sub>2</sub>PO<sub>4</sub>, 10 mM glucose and 5 mM HEPES (pH, 7.4) with 100% O<sub>2</sub> at 37°C. Single atrial myocytes were obtained by the dispersion method as previously described.<sup>10</sup>

Overall, it took 1 hour to isolate the cells. Many viable cells were isolated from each of the 3 regions but only 1 to 2 cells were used for the patch-clamp technique, which took about 2 hours.

The whole-cell configuration of the patch-clamp technique was used in this study. The isolated cells were perfused with the Tyrode solution containing 136 mM NaCl, 5.4 mM KCl, 1 mM CaCl<sub>2</sub>, 1 mM MgCl<sub>2</sub>, 10 mM glucose, and 10 mM HEPES (pH, 7.4). The pipette solution was composed of 110 mM potassium aspartate, 20 mM KCl, 1 mM MgCl<sub>2</sub>, 5 mM Mg-ATP, 0.1 mM GTP, 10 mM EGTA, 5 mM phosphocreatine, 10 mM HEPES, and the pH was adjusted to 7.3 with KOH. Command pulses were generated by a converter controlled by Pulse/Pulsefit software (Heka Instruments, Germany). Junction potentials were set to zero before the formation of the membrane-pipette seal in the Tyrode solution. The capacitance and series resistance were both electrically compensated to minimize the duration of the capacitive surge on the current recording and the voltage drop across the clamped cell membrane. Cells with changing leak current (indicated by changes of more than 10 pA in the holding current at -50 mV) were rejected.

Experiments were conducted at 32°C.

To record I<sub>KACh</sub>, other subtypes of muscarinic cholinergic receptors were inhibited using the subtype-selective antagonists pirenzepine (100 nM, an M<sub>1</sub> blocker), 4-DAMP (2 nM, an M<sub>3</sub> inhibitor) and tropicamide (200 nM, an M<sub>4</sub> inhibitor). This is the only I<sub>KACh</sub> change marked by the authors. I<sub>KACh</sub> was induced by ACh (1 µM) in the bathing solution and defined as the atropine (1 µM)-sensitive current to rule out contamination from the background inward rectifier K+ current (I<sub>K1</sub>).
Changes in AERP and I\textsubscript{KACH} After Vagal Stimulation

Changes in the Atrial Effective Refractory Period and in the Dispersion of the Atrial Effective Refractory Period

In the pacing groups, the AERP was markedly shorter at all sites and the dAERP was significantly increased (11 [3] ms vs 32 [5] ms; \(P<.05\)), respectively. However, in the VS-plus-pacing group, the AERP was not significantly changed after pacing, whereas the dAERP also increased significantly (10 [3] ms vs 30 [5] ms; \(P<.05\)) (Tables 2 and 3).

### Statistical Analysis

Values are expressed as means plus or minus the standard error of the mean. The SPSS statistical software package was used for analysis. The statistical comparisons were performed with ANOVA. The comparisons of paired and of unpaired data were carried out using the Student \(t\) test and incidences of AF were compared by Fisher’s exact test. Statistical significance was assumed if \(P\) values were less than .05.

### RESULTS

#### Induction of Atrial Fibrillation

In the control group, AF was induced in 1 of the 8 animals. The duration of AF was 5 seconds. In the pacing group, AF was induced in all 8 animals and its duration was over 10 seconds. In the VS-plus-pacing group, AF was induced in 2 animals. The duration of AF was 4 seconds in 1 animal and 5 seconds in the other. The incidence of induced AF was higher and its duration longer in the pacing group than in the control and the VS-plus-pacing groups (\(P<.05\)); however, there were no significant differences between the control group and the VS-plus-pacing group (Table 1).

#### Correlation Between Pacing and I\textsubscript{KACH} Density

The amplitude of I\textsubscript{KACH} was measured as an average of the currents at the end of the two-second voltage steps after the onset of these voltage steps. As illustrated, in the control group, the densities of I\textsubscript{KACH} were substantially lower in the LSPV cells than those observed in the atrial myocytes at all the potentials tested. Furthermore, the I\textsubscript{KACH} densities were lower in the right atrial myocytes (LA, RA vs LSPV: -14 [0.58], -10 [0.63] vs -7 [0.42] pA/pF; \(P<.05\)). In the pacing group, the densities of I\textsubscript{KACH} were increased at all sites (LSPV: -17 [0.61] vs -14 [0.58] pA/pF; LA: -13 [0.57] vs -10 [0.63] pA/pF; RA: -11 [0.53] vs -7 [0.42] pA/pF).

### TABLE 1. Characteristics of Atrial Fibrillation Induction

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Pacing</th>
<th>VS+Pacing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of animals</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Number sustained AF (&gt;5 s)</td>
<td>0</td>
<td>8*</td>
<td>0</td>
</tr>
<tr>
<td>Mean duration of AF, s</td>
<td>0.625</td>
<td>14*</td>
<td>1.125</td>
</tr>
<tr>
<td>Longest duration of AF, s</td>
<td>5</td>
<td>17*</td>
<td>5</td>
</tr>
</tbody>
</table>

*Pacing versus control and VS+pacing, \(P<.01\)
AF indicates atrial fibrillation.

### TABLE 2. Changes in Atrial Effective Refractory Period Before and After Vagal Stimulation in the Pacing and Vagal Stimulation-Plus-Pacing Groups

<table>
<thead>
<tr>
<th>AERP, ms</th>
<th>LSPV</th>
<th>LA</th>
<th>RA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pacing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before pacing, mean (SEM), ms</td>
<td>133 (6)</td>
<td>142 (7)</td>
<td>141 (7)</td>
</tr>
<tr>
<td>After pacing, mean (SEM), ms</td>
<td>101 (8)*</td>
<td>119 (10)*</td>
<td>114 (9)*</td>
</tr>
<tr>
<td>VS+pacing group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before VS\textsubscript{2}+pacing, mean (SEM), ms</td>
<td>136 (6)</td>
<td>143 (8)</td>
<td>140 (7)</td>
</tr>
<tr>
<td>After VS\textsubscript{2}+pacing, mean (SEM), ms</td>
<td>124 (11)</td>
<td>139 (12)</td>
<td>139 (12)</td>
</tr>
</tbody>
</table>

*Before versus after VS\textsubscript{2}, \(P<.01\); before versus after pacing, \(P<.05\).
Table 2 shows that AERP was shortened at all sites after VS or pacing in pacing group. However, there was no significant difference in AERP before and after pacing in VS+pacing group. LSPV pacing was applied at 8.33 Hz, VS\textsubscript{1} at 20 Hz, and VS\textsubscript{2} at 5 Hz.
AERP indicates atrial effective refractory period; LA, left atrium; LSPV, left superior pulmonary vein; RA, right atrium; SEM, standard error of the mean; VS, vagal stimulation.
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TABLE 3. Changes in Dispersion of Atrial Effective Refractory Period Before and After Pacing (Vagal Stimulation1) in the Pacing and Vagal Stimulation-Plus-Pacing Groups

<table>
<thead>
<tr>
<th></th>
<th>Before Pacing (or VS)</th>
<th>After Pacing (or VS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pacing group, mean (SEM), ms</td>
<td>11 (3)</td>
<td>32 (5)*</td>
</tr>
<tr>
<td>VS+pacing group, mean (SEM), ms</td>
<td>10 (3)</td>
<td>30 (5)*</td>
</tr>
</tbody>
</table>

*Before versus after VS, $P<.05$.

Table 3 shows that dAERP increased significantly after pacing or VS in the pacing and VS+pacing groups.

dAERP indicates dispersion of atrial effective refractory period; SEM, standard error of the mean; VS, vagal stimulation.

DISCUSSION

The results of the present study show that: a) prior to rapid pacing, VS can inhibit the vulnerability to AF, and b) rapid burst pacing in LSPV increases the densities of I\textsubscript{K\textsubscript{ACH}} in the atrium and LSPV, while VS prior to pacing inhibits the changes in I\textsubscript{K\textsubscript{ACH}}. These results suggest that the effect of rapid pacing on atrial electrical remodeling is related to an increase in the I\textsubscript{K\textsubscript{ACH}}.

Recent studies have suggested that the vagal nerve plays an important role in the development of and recovery from atrial electrical remodeling associated with rapid pacing.\textsuperscript{5,7} This study indicates that an increase in vagal tone together with electrical remodeling might act synergistically to shorten the refractory period and promote AF. Similarly, Miyauchi et al\textsuperscript{6} showed that parasympathetic blockade with atropine promoted recovery from atrial electrical remodeling induced by short-term atrial pacing in humans. However, Takei et al\textsuperscript{7} demonstrated that vagal stimulation prior to rapid atrial pacing prevented electrical remodeling. Perhaps the different findings indicate that the vagal tone has different effects on the electrical remodeling before and after rapid atrial pacing.

Data from other studies have demonstrated a marked shortening of the APD and formation of early after-depolarizations in superfused pulmonary vein sleeves when exposed to acetylcholine and norepinephrine or with local electrical stimulation.\textsuperscript{12,13} Rapid activations within the pulmonary veins are important in the mechanisms of AF. The LSPV is a frequent source of these rapid activations during AF.\textsuperscript{14} To investigate the effect of VS on electrical remodeling before rapid LSPV pacing, we observed different changes in the AERP after VS plus rapid LSPV pacing. We found that after pacing without VS, there was a sharp decrease in the AERP and a significantly increased dAERP. However, after VS plus pacing, the AERP did not
In the present study, the results showed that increases in the dAERP alone were not enough to induce AF but, rather, the decrease in the AERP, was the basis of the initiation of AF.

Several studies have demonstrated different distributions of $I_{K_{ACh}}$ in the atrium and pulmonary veins. Chronic atrial tachycardia in the range of that of AF produces important alterations in ion channel function (reduced densities of transient outward K$^+$ current $I_{to}$, L-type Ca$^{2+}$ current $I_{Ca,L}$ and Na$^+$ current $I_{Na}$) that result in a functional substrate that supports the maintenance of AF. In chronic human AF, Dobrev et al. showed that down regulation of $I_{K_{ACh}}$ attenuates the muscarinic receptor–mediated shortening of APD. Furthermore, in their other study, they demonstrate that larger basal inward rectifier K$^+$ current in chronic AF consists of increased $I_{K1}$ activity and constitutively active $I_{K_{ACh}}$. These results showed that the shortening of the AERP due to electrical remodeling was counteracted by down regulation of $I_{K_{ACh}}$.

In the present study, we observed the densities of $I_{K_{ACh}}$ at different sites and under different conditions. The results showed that, after pacing, the densities of $I_{K_{ACh}}$ were increased in LSPV, LA and RA. The mechanisms by which the densities of $I_{K_{ACh}}$ change with rapid atrial pacing or sustained AF are unknown. Dobrev et al. suggested that atrial myocytes adapt to a chronically high rate by downregulating $I_{K_{ACh}}$ to counteract the shortening of the AERP due to electrical remodeling. However, our data showed that, after 4 hours of rapid pacing, densities of $I_{K_{ACh}}$ were increased. After VS plus pacing, we observed that there were no differences in $I_{K_{ACh}}$ between sinus rhythm and after VS prior to pacing. This remodeling of $I_{K_{ACh}}$ may explain why VS plus pacing protected the atrium from atrial electrical remodeling. In our previous study, we found that rapid pulmonary vein pacing induced a decrease in $I_{Ca,L}$ and $I_{to}$ densities. To our knowledge, the essential elements required for this process are currently unknown. A recent study showed that $I_{to}$ in rabbit atrium is depressed after short-time rapid atrial pacing but recovers after a longer pacing period. The time course of $I_{K_{ACh}}$ remodeling when oscillations are produced should be further investigated.

Limitations of the Study

Pentobarbital is known to prolong the AERP as compared to the unanesthetized state and it affects sympathetic and parasympathetic tone,
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which may be a limitation in the present study. All the dogs were self-controlled and received the same dose as well as the same kind of anesthetic, and pentobarbital sodium has little effect on the autonomic nerve as compared with VS. In our study, we observed the changes in the AERP and IKACH after only 4 hours of pacing. The effect of long-term pacing on IKACH may yield different results and should be further investigated.

Furthermore, we did not examine the activity of IKACH after VS (5 Hz frequency, with a 0.2 ms pulse duration and at a voltage of 5-10 V) for 30 minutes, and continuous VS during rapid pacing might have yielded different results. Finally, this study addressed neither the question of the time course necessary to influence IKACH nor the hemodynamic variables during rapid pacing. However, there were no significant differences in peripheral edema or skin temperature between the 2 groups. Future studies should investigate whether the vagal tone influences IKACH and the effect of hemodynamic variables on vagal tone.

CONCLUSION

Decreased AERP may be fundamental, but is not the only cause for the cholinergic induction of AF. Rapid LSPV pacing can increase IKACH; however, VS prior to rapid pacing partially protects the atria from electrical remodeling.

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