Radiofrequency Ablation at the Coronary Sinus Ostium Interrupts the Vagal Efferent Input to the Atrioventricular Node in the Canine Heart

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The fat pad at the junction of the inferior vena cava and inferior left atrium is the area of convergence of vagal projections into the atrioventricular node (AVN) region. The present study investigated whether radiofrequency (RF) ablation applied to the area around the coronary sinus (CS) ostium would impair vagal input to the AVN in the canine heart. Twenty-four dogs were anesthetized by sodium pentobarbital and RF energy was delivered at 20 W for 5–10 s. In the baseline state without vagal stimulation (10 Hz, 2 ms), the electrophysiological variables did not change significantly after RF ablation. Vagally induced changes in the sinus cycle length and effective refractory period of the right atrium and left ventricle did not differ after RF ablation. However, the effects of vagal stimulation on the AVN function were impaired after RF ablation to the CS area from the ostium to 10 mm within the ostium. After ablation was applied to the fast pathway area, the vagally induced changes in the AVN function decreased, but these changes were not affected after RF ablation in the slow pathway area. RF ablation in the vicinity of the CS would attenuate vagal input to the AVN. (Jpn Circ J 2001; 65: 667–672)

Key Words: Atrioventricular node; Coronary sinus; Denervation; Radiofrequency ablation; Vagal efferent fiber

Persistent inappropriate sinus tachycardia is seen after radiofrequency (RF) ablation of the fast or slow pathway in patients with atrioventricular nodal reentrant tachycardia (AVNRT).1,2 The disruption of vagal nerves destined to innervate the sinoatrial node (SAN) is thought to result in this arrhythmia,2–5 but divergent results have been reported; for example, an increase or preservation of vagal tone to the SA node after RF ablation has been reported.6 Shortening of the effective refractory period (ERP) of the fast pathway after RF ablation of the slow pathway is not mediated by autonomic changes,7 and vagal innervation to the atrioventricular node (AVN) is unchanged after RF ablation of the slow pathway.8–10 RF ablation of ventricular tachycardia could result in alteration of cardiac autonomic tones determined by heart rate variability.11

The fat pad at the junction of the inferior vena cava and inferior left atrium (IVC-ILA) is an area of convergence of vagal projections into the AVN region. The purpose of the present study was therefore to determine whether application of RF energy to the area around the coronary sinus (CS) would alter vagal efferent innervation to the SAN, AVN, atrium and ventricle in the canine heart.

Methods

All experiments were performed in accordance with the ‘Guideline for Animal Experiment’ at Toyama Medical and Pharmaceutical University.

Surgical Procedures

Twenty-four mongrel dogs of either sex, weighing 8–13 kg, were anesthetized with intravenous sodium pentobarbital at a dose of 30 mg/kg. Additional amounts of pentobarbital were injected as needed to maintain anesthesia. No measurements were taken for at least 15 min after pentobarbital administration. The dogs were intubated with an endotracheal tube and artificially ventilated with room air using a volume-cycled respirator (Harvard Model 607, Chicago, IL, USA). A femoral vein cannula was used to infuse normal saline at a rate of 100–200 ml/h to replace spontaneous fluid losses. The chest was opened through a median sternotomy, and the heart was suspended in a pericardial cradle. The thoracotomy was covered by a plastic sheet, and an operating lamp was used to maintain the temperature of the chest.

The bilateral, cervical vagus nerves were isolated, doubly ligated and cut in all dogs. Two Teflon-coated wire electrodes were embedded in the cardiac end of each vagus nerve to stimulate the efferent fibers. The ansae subclaviae were also isolated where they exited from the stellate ganglia, doubly ligated and cut. Bipolar hook electrodes, with an interhook distance of approximately 1 mm, were placed in the middle right atrium and anterior left ventricle, and used to determine atrial and ventricular activation. Electrodes that would serve as a cathode for unipolar stimulation were placed in the high right atrium and posterior left ventricle. The anode was placed in the abdominal wall. A 6Fr bipolar catheter (USCI, Billerica, MA, USA) was advanced through the right carotid artery into the noncoronary cusp of the aortic valve to record the His bundle electrogram. Atrial, His bundle, and ventricular electrograms filtered at 50–300 Hz were recorded simultaneously with the surface electrocardiographic lead II on a thermal recorder (RTA-1200M, Nihon Kohden, Tokyo, Japan) at a paper
speed of 100 mm/s and stored in a digital data recorder (RD-130TE, TEAC, Tokyo, Japan) for later analyses. A storage oscilloscope was also used to monitor the ECG and the atrial and ventricular electrograms.

Measurements of Electrophysiological Variables
Sinus cycle length (SCL) was measured from the electrogram recorded with the bipolar hook electrode in the middle right atrium. The ERP was determined at each test site by the extravascular stimulus technique using a digital programmable stimulator (SEC-202, Nihon Kohden). Each test site was driven by a 2-ms rectangular stimulus at twice the diastolic threshold. The late diastolic thresholds of the test sites were measured during each intervention. A train of 8 stimuli (S1) at a constant cycle length of 200 ms was followed by a late extrastimulus (S2) that produced a propagated atrial or ventricular response. The atrial or ventricular response to S2 was obtained from the bipolar electrode in the right atrium or the left ventricle and displayed on the storage oscilloscope. The S1–S2 interval was shortened in steps of 2 ms until S2 failed to produce a propagated response. The ERP was defined as the longest S1–S2 interval at which S2 failed to produce a propagated response. The measurements were repeated to ascertain the reproducibility of the ERP.

The Wenckebach cycle length (WCL) and AVN conduction curve were determined to analyze the functional properties of the atrioventricular (AV) conduction system. Incremental right atrial pacing was carried out to detect the WCL, defined as the longest atrial-paced cycle length at which a gradual prolongation of the succeeding AV conduction interval occurred. Each pacing cycle length was shortened in steps of 10 ms every 15 s from a cycle length just below sinus rhythm. A single atrial extrastimulus was delivered after a train of 8 atrial stimuli at a basic cycle length of 600 ms to determine the AVN conduction curve, which was drawn by plotting the atrial coupling intervals of A1–A2 to the responses of the coupling intervals of His deflection (H1–H2). The minimum H1–H2 interval was defined as the functional refractory period (FRP) of the AVN.

Vagal Nerve Stimulation
Stimulation of the right and left vagus nerves was performed with separate isolated constant-current sources (SS-202J, Nihon Kohden), driven by a programmable stimulator (SEN-7103, Nihon Kohden). The current strength was set to prolong the SCL by 100% with right vagal stimulation and to produce a 2:1 AV block with left vagal stimulation by 2-ms rectangular pulses at 20 Hz. In some dogs, left vagal stimulation produced complete AV block without producing a 2:1 block. In these dogs, the current strength that produced complete AV block was used. For determination of electrophysiological variables, the vagus nerves were both stimulated at 10 Hz. Between interventions, the nerves were stimulated to be certain that the SAN and AVN responses remained constant.

Experimental Protocol
After determining the baseline values, the vagus nerves were both stimulated and the electrophysiological variables were determined. Then RF ablation was applied using a 7Fr deflectable catheter with a large (4 mm) tip (EP Technology Inc. Sunnyvale, CA, USA). The catheter was inserted through the right femoral vein or the right cervical vein and advanced to the area adjacent to the CS ostium. RF energy at 20 W was applied for 5–10 s using a RF coagulator (CAT-500, Central Kogyo, Chiba, Japan). The RF energy was delivered at 500 kHz between the large tip and a copper plate (90 × 90 mm) placed in the abdominal wall. When an abrupt impedance rise (>30 ohms from the baseline level) occurred, energy application was stopped automatically. After the application of RF energy, digital data including current output, voltage output, total energy, and impedance were displayed. The electrophysiological variables were determined as before RF ablation.

Anatomical and Histological Studies
After the experiment, the heart was removed and the free wall of the right atrium was resected to ascertain the ablated site. The slow pathway area was identified as the region that coursed along the tricuspid annulus in the lower half of the triangle of Koch and the fast pathway area was identified as the region that coursed along the tendon of Todaro. When the RF energy was applied to the CS, the distance between the CS ostium and the proximal end of the ablation site was determined.

The fat pad at the IVC-ILA junction, the fat tissue along the AV groove and the underlying myocardium were excised (Fig 1) and fixed in 10% formaldehyde solution. To determine the distribution of vagal ganglia around the crux, the fat tissue in 5 additional dogs that did not receive RF ablation was examined. Fat tissue with underlying myocardium was cut to obtain 6 blocks of 10 mm in height and 5 mm in width along the AV groove (Fig 1). The excised blocks were named L1, L2 and L3, with L1 being the block nearest to the crux along the left AV groove, and R1, R2 and R3, with R1 being the block nearest to the crux along the right AV groove. Each block was sliced parallel to the AV groove and perpendicular to the endocardial surface for microscopic examination. Five serial sections (15 μm thick) were taken at every 1 mm (ie, 25 slices per block), and stained with hematoxylin–eosin for microscopic examination of the presence and distribution of vagal ganglia in each block of fat tissue. The mean number of ganglia per section was compared between the 6 sites.
Statistical Analysis

The data are expressed as mean±SD. Statistical analyses of the electrophysiological data between before and after vagal stimulations, and between before and after RF ablation were performed by Student’s paired t test. The difference between the mean numbers of vagal ganglia was determined by using analysis of variance (ANOVA) for repeated measures. Multiple comparisons were made by Fisher’s method, if indicated. The relation between 2 variables was tested with a linear correlation analysis. The statistical significance level was set at p<0.05.

Results

Electrophysiological Variables Before and After RF Ablation

The vagally induced changes in the electrophysiological variables are summarized in Table 1. The current strength for right vagal stimulation was 0.15±0.10 mA and did not differ from that for left vagal stimulation (0.22±0.13 mA). The SCL was lengthened significantly after vagal stimulation; other electrophysiological variables changed as expected after vagal stimulation13. The vagally induced changes in these variables after RF ablation are summarized in Table 2. The current strength used did not differ significantly between before and after RF ablation. In the baseline state without vagal stimulation, the electrophysiological variables after ablation did not change significantly from those before ablation. The vagally induced changes of SCL and ERP of the right atrium and the left ventricle did not differ from those before ablation (Table 2).

The changes in WCL and FRP by vagal stimulation differed depending on the ablation site: the CS ostium and in the CS in 16 dogs, the slow pathway area in 6 dogs, and the fast pathway area in 2 dogs (Fig 2). The number of applications of RF energy and the total energy did not differ between the 3 ablation sites (detailed later). When RF ablation was attempted at the CS ostium and in the CS (2.1±1.2 applications with a total energy of 277±174 J), the vagally induced changes in the WCL and FRP of the AVN were attenuated (Table 3), and the AV conduction curve shifted to the left and downward (Fig 3) during vagal stimulation. When RF ablation was attempted in the fast pathway area parallel to the left border of Koch’s triangle (2.5±0.7 applications with a total energy of 377±181 J), the changes in the AV conduction properties were similar to those after ablation of the CS and its ostium (Table 3). When RF ablation was attempted in the slow pathway area in the lower half of Koch’s triangle along the tricuspid valve (1.8±1.0 applications with a total energy of 311±180 J), the vagally induced changes in the WCL and FRP of the AVN did not change significantly (Table 3) and the AV conduction curve did not shift significantly (Fig 4) during vagal stimulation.

The relation between the distance of the ablation site in the CS from the CS ostium and the changes in the WCL after RF ablation is shown in Fig 5; a significant (p<0.01) positive linear correlation (r=0.67) was found and was also true for changes in the vagally induced lengthening of the FRP after ablation (r=0.70, p<0.01). This indicates that the

Table 1 Changes in the Electrophysiological Variables by Vagal Stimulation Before Radiofrequency Ablation

<table>
<thead>
<tr>
<th>Variable (ms)</th>
<th>Baseline</th>
<th>Vagal stimulation</th>
<th>p value</th>
</tr>
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<tbody>
<tr>
<td>SCL</td>
<td>481±90</td>
<td>902±191</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>ERP (RA)</td>
<td>152±24</td>
<td>129±19</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>ERP (LV)</td>
<td>176±23</td>
<td>183±22</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>WP</td>
<td>242±41</td>
<td>457±113</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>FRP</td>
<td>286±68</td>
<td>432±46</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD. SCL, sinus cycle length; ERP, effective refractory period; WP, Wenckebach point; FRP, functional refractory period of the atrioventricular node; RA, right atrium; LV, left ventricle.

Table 2 Changes in the SCL, ERP, WP and FRP by Vagal Stimulation After Radiofrequency Ablation

<table>
<thead>
<tr>
<th>Variable (ms)</th>
<th>Baseline after ablation</th>
<th>Vagal stimulation</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCL</td>
<td>520±93</td>
<td>928±208</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>ERP (RA)</td>
<td>153±39</td>
<td>131±22</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>ERP (LV)</td>
<td>182±24</td>
<td>189±23</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>WP</td>
<td>266±45</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>FRP</td>
<td>293±58</td>
<td>*</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD. Abbreviations as in Table 1. *WP and FRP during vagal stimulation are not shown in this Table because the changes differed depending on the ablation site.
greater the distance between the ablation site and the CS ostium, the lesser the effect on vagal efferent input to the AVN.

Histological Findings

Autonomic ganglia that varied in size and in number from 2 to 20 were found in each block of fat tissue. Each ganglion contained small clusters of cells that were often embedded in nerve branches and the clusters near the periphery of the myocardium were larger than those at a distance from the myocardium. Ganglia were present within the connective tissue between muscle bundles, but not in the atrial endocardium. These findings were consistent with those of a previous study.14

Table 3  Effects of Radiofrequency Ablation on the WP and FRP During Vagal Stimulation

<table>
<thead>
<tr>
<th></th>
<th>Pre-ablation</th>
<th>Post-ablation</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coronary sinus and its ostium (n=16)</td>
<td></td>
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<tr>
<td>WP (ms)</td>
<td>454±112</td>
<td>368±87</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>FRP (ms)</td>
<td>300±51</td>
<td>230±47</td>
<td>&lt;0.01</td>
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<tr>
<td>Fast pathway area (n=2)</td>
<td></td>
<td></td>
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<tr>
<td>WP (ms)</td>
<td>498±127</td>
<td>295±144</td>
<td></td>
</tr>
<tr>
<td>FRP (ms)</td>
<td>385±60</td>
<td>280±105</td>
<td></td>
</tr>
<tr>
<td>Slow pathway area (n=6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WP (ms)</td>
<td>412±95</td>
<td>440±78</td>
<td>NS</td>
</tr>
<tr>
<td>FRP (ms)</td>
<td>321±26</td>
<td>325±31</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD. Abbreviations as in Table 1.
The ganglia were most abundant in L1 adjacent to the endocardially located CS ostium compared with the other blocks examined along the AV groove (Fig 6). The number of ganglia gradually decreased from L1 to L3. The number of ganglia along the left AV groove was greater than that along the right AV groove; in particular, ganglia were scarce in R2 and R3.

The histological examination revealed that in the dogs which had received RF energy to the CS ostium or in the CS, there was necrosis of the fat tissue surrounding the ganglia and nerve branches, and a wavy pattern in the myocardium adjacent to the fat tissue.

**Discussion**

The major findings of this study are as follows. First, in the baseline state, the electrophysiological values were not changed significantly by RF ablation in the area around the CS ostium. Second, changes in the SCL and ERP of the right atrium and the left ventricle induced by vagal stimulation were not altered after RF ablation in the area around the CS. Third, the efferent vagal input to the AVN was impaired by RF ablation in the CS ostium and the fast pathway area.

**Alteration of Autonomic Input After RF Ablation**

Persistent inappropriate sinus tachycardia of more than 100 beats/min is a well-known complication after RF ablation of the fast pathway in patients with AVNRT. Inappropriate sinus tachycardia has also been seen after RF ablation of the slow pathway and a posteroseptal accessory pathway, but not of a left lateral accessory pathway. Sympathetic predominance is not likely to be the mechanism of inappropriate sinus tachycardia after RF ablation may be vagal denervation in the interatrial septum and the slow pathway area. Frey et al reported that denervation of the SAN and AVN after selective RF ablation was associated with an alteration of the slow pathway area.

There are diverse results about the effects on the vagal innervation of the AVN by RF ablation of the slow pathway. Kowallik et al determined whether successful RF ablation of AVNRT was associated with an alteration of the autonomic input to the SAN and AVN by analyzing the power spectra of the beat-to-beat PP and PR intervals, and they found that autonomic modulation of both nodes was preserved following RF ablation. Kautzner et al showed that reflex vagal stimulation with phenylephrine did not significantly influence AVN conduction, as assessed from the AH interval, which suggests that RF ablation of the slow pathway does not interrupt vagal innervation to the AVN. Olsovsky et al failed to obtain evidence of vagal denervation of the SAN and AVN after selective RF ablation of the slow pathway.

Moreover, RF ablation can have opposite effects to those reported by the aforementioned studies of denervation. Frey et al reported an increased heart rate variability immediately after RF ablation of a posteroseptal accessory pathway and the increase correlated with the amount of cumulative energy applied, consistent with either a far-field effect on the SAN or increased vagal tone. A case associated with asystole (Bezold-Jarisch-like phenomenon) induced by RF ablation of a left posteroseptal accessory pathway has been reported and was possibly caused by stimulation of efferent parasympathetic pathways linked with sensory endings in the inferoposterior myocardial wall.

The present study demonstrated that RF ablation to the area around the CS ostium could, at least in part, interrupt the efferent vagal input to the AVN, but not the SAN.

**Autonomic Nervous System in the Canine Heart**

There is a clear understanding of the cardiac autonomic innervation in the dog. Postganglionic sympathetic and preganglionic parasympathetic fibers combine at the base of the heart to form the cardiac plexus with its dorsal and ventral parts. The pathways of the autonomic nerves to the canine heart have been determined using the techniques of electrical stimulation and surgical intervention. The vagal pathways enter the SAN along the superior vena cava (SVC), superior left atrium, and interatrial groove, and the AVN along the IVC-ILA junction and the superior left atrium but do not course from left to right along the AV groove.

The intrapericardial projections of the left vagus to the SAN and AVN penetrate the epicardium in the region of the common pulmonary vein (PV) complex. In contrast, the intrapericardial projections of the right vagus are more diffuse, but appear to penetrate the epicardium adjacent to the first bifurcation of the right pulmonary artery (PA) and the origin of the right PV. A triangular fat pad overlaying the junction of the right PV and the right atrium (PVP) contains both the right and left vagal ganglia innervating the SAN and the bilateral vagal input to the AVN is via a triangular fat pad at the IVC-ILA junction. These fat pads contain multiple, well-organized autonomic ganglia that are surrounded by connective tissue closely adjacent to epicardial muscle. Most efferent vagal fibers travel through the fat pad located between the medial SVC and the aortic root, and then project into the PVFP and IVC-ILA fat pads, which provide the vagal innervation to the SAN and AVN, respectively.

We have shown in the present study that the efferent vagal input to the AVN was attenuated by RF ablation in the CS ostium. The IVC-ILA fat pad contains the vagal ganglia innervating the AVN, so our finding suggests that the IVC-ILA fat pad, vagal ganglia and the postganglionic vagal fibers are injured by the RF energy applied from the endocardial side. The vagally induced changes in the SCL and ERP of the right atrium and left ventricle were not altered significantly after RF ablation.

The route of the neural fibers from the vagal ganglia to the AVN is unclear. In the present study, we found that the number of ganglia decreased gradually from the CS ostium along the left AV groove, and RF ablation in the fast pathway area could damage the vagal fibers innervating the AVN. However, vagal input to the AVN was not interrupted by RF ablation in the slow pathway area. Therefore, the slow pathway area may be separate from both the vagal ganglia and neural fibers.

**Study Limitations**

First, direct application of information derived from this experimental study to patients is not warranted because the comparable innervation of the human heart has not been demonstrated yet. Second, we determined only the acute effects of ablation and it is unknown whether re-innervation occurs during a long term follow-up? Third, most of the ablation sites were in the CS or around its ostium and ablation in the so-called fast pathway area was only
attempted in 2 dogs. Therefore, the results of RF ablation in the so-called fast pathway area should be interpreted carefully. Fourth, we stimulated both vagal nerves electrically with a non-physiologic current strength and the criteria for selection of current strength for vagal stimulation differed between the right and left vagus. However, the current strength did not differ significantly between the right and left vagus, and did not differ significantly between before and after RF ablation.

Clinical Implications

Although caution is required when extrapolating the experimental data to clinical patients, our study has some clinical relevance. Selective RF ablation in the slow pathway area might be safer in terms of damage to the vagal input of the AVN, whereas RF energy applied to the fast pathway area and CS ostium could disturb vagal input to the AVN activity.

Acknowledgment

This study was supported by a Research Grant for Cardiovascular Disease (6C-1), from the Ministry of Health and Welfare of Japan.

References