Changes in Atrial and Ventricular Refractoriness and in Atrioventricular Nodal Conduction Produced by Combinations of Vagal and Sympathetic Stimulation That Result in a Constant Spontaneous Sinus Cycle Length

Hiroshi Inoue and Douglas P. Zipes

The vagal nerves modulate adrenergic effects on sinus cycle length, atrioventricular (AV) nodal conduction, and refractoriness of atria and ventricles. We tested whether varying levels of vagal-sympathetic input could yield the same spontaneous sinus cycle length but also alter effective refractory periods of the right atrium, right ventricle, and left ventricle and AV nodal conduction times. Dogs anesthetized by α-chloralose were studied in the open-chest, neurally decentralized state. In 10 dogs, sinus cycle length was maintained constant during levels of bilateral ansa subclaviae stimulation (4 msec, 3 mA at 1, 2, and 4 Hz) by titrating simultaneous bilateral vagal stimulation (varying pulse width and frequency). Each combination of ansa subclaviae-vagal stimulation yielded the same sinus cycle length as the control value, but refractory periods of right atrium and right and left ventricles shortened progressively as the frequency of ansa subclaviae stimulation increased. Atrioventricular nodal conduction time (AH interval) shortened in 2 dogs and lengthened in 3 dogs. His-Purkinje conduction time (HV interval) was unchanged. In 9 dogs, the effects of simultaneous unilateral ansa subclaviae stimulation at 2 Hz and ipsilateral vagal stimulation that yielded the same sinus cycle length were determined. Right-sided ansa subclaviae-vagal stimulation shortened refractoriness of right atrium and anterior left ventricle significantly. The AH interval lengthened in 1 dog. Left ansa subclaviae-vagal stimulation shortened the refractory periods of anterior and posterior left ventricle significantly and reduced the AH interval in 3 dogs. In 8 dogs, the effects of bilateral ansa subclaviae stimulation alone at 2 Hz, vagal stimulation alone at an intensity required to keep the sinus cycle length constant during ansa subclaviae stimulation, and simultaneous bilateral ansa subclaviae and vagal stimulation were tested. The right atrial refractory period was shortened significantly by ansa subclaviae stimulation alone and by vagal stimulation alone and was shortened further by simultaneous stimulation of both autonomic limbs. The right and left ventricular refractory periods were shortened by ansa subclaviae stimulation alone and by simultaneous stimulation of both limbs but tended to be prolonged by vagal stimulation alone and when added to ansa subclaviae stimulation. In 7 dogs, the effects of simultaneous stimulation of bilateral ansa subclaviae at 2 Hz and vagal at intensities that maintained the AH interval constant at an atrial pacing cycle length of 300 msec were determined. During these stimulation parameters, the sinus cycle length was prolonged by 90 msec or more in 3 dogs and varied within 15 msec in the remaining 4 dogs, while refractoriness of atrium and ventricle shortened significantly. We conclude that multiple combinations of ansa subclaviae-vagal stimulation that do not alter the spontaneous sinus cycle length can lengthen or shorten AV nodal conduction time and can shorten atrial and ventricular refractory periods. Under these conditions, sinus cycle length is not an indicator of autonomic input to the AV node, atrium, and ventricle of the canine heart. Similarly, under autonomic conditions that yielded a constant AH interval, atrial and ventricular refractory periods shortened and spontaneous sinus cycle length lengthened or shortened. (Circulation Research 1987;60:942-951)

Although both limbs of the autonomic nervous system modulate cardiac electrophysiologic properties, they demonstrate dominance in different areas of the heart and on different electrophysiologic properties, including chronotropy, dromotropy, and excitability. At the sinus node, the vagus predominates, and during tonic adrenergic discharge, vagal stimulation produces a greater absolute increase in the sinus cycle length than vagal stimulation during minimal adrenergic activity. This sympathetic-vagal interaction has been called "accentuated antagonism." Such vagal modulation of sympathetic effects probably results from interactions at prejunctional and postjunctional sites. Interestingly, phasic vagal stimulation does not produce accentuated antagonism. Stimulation of both autonomic limbs shortens atrial refractoriness, although vagal stimulation has an effect that is more pronounced than sympathetic stimulation. At the atrioventricular (AV) node, vagal and sympathetic effects are opposite and "algebraically ad-
The spontaneous sinus rate often is used to reflect the level of autonomic tone to the rest of the heart. Based on the above reasoning, however, an increase in sympathetic effects are more important. Naturally, dysfunction of one or the other autonomic limb or an abnormal response of the sinus node to autonomic input increases the possibilities that the sinus cycle length might be a poor "autonomic barometer."

The purpose of this study was to test the hypothesis that varying levels of sympathetic and vagal input could both yield the same spontaneous sinus cycle length, and alter atrial and ventricular effective refractory periods and AV nodal conduction in the canine heart.

Materials and Methods

Surgical Procedures

Twenty-four mongrel dogs of either sex, weighing 15–27 kg, were anesthetized with 100 mg/kg α-chloralose i.v. Additional amounts of chloralose (10 mg/kg/hr) were injected as needed to maintain anesthesia. No measurements were obtained for at least 15 minutes after chloralose administration. The dog was intubated and ventilated with room air using a Harvard Model 607 volume-cycled respirator. The chest was opened through a median sternotomy, and the heart was suspended in a pericardial cradle. A fluid-filled cannula placed in the femoral artery was connected to a Statham p-23Db transducer to monitor arterial blood pressure, and a femoral venous cannula was used to infuse normal saline at 100–200 ml/hr to replace spontaneous fluid losses. The dog was placed on a heating pad, and the thoracotomy was covered by a plastic sheet. An operating table lamp was used to maintain epicardial temperature between 37 and 39°C. Arterial blood gases and pH were monitored and maintained within the physiologic range.

The cervical vagi were isolated, doubly ligated, and cut in all dogs. Two Teflon-coated wire electrodes were embedded in the cardiac end of each vagal nerve to stimulate efferent vagal nerves. The ansae subclaviae were isolated as they exited from the stellate ganglia, doubly ligated, and cut. Shielded bipolar electrodes were placed on the right and left anterior and posterior ansae subclaviae to stimulate the efferent cardiac sympathetic nerves. The sinus node was not crushed. Hook electrodes made from Teflon-coated wires, insulated except for their tips, were placed in the high and low right atrium, anterior right ventricle, and anterior and posterior left ventricle using a 22-gauge needle. The electrodes served as a cathode for unipolar stimulation. The anode was a 33-mm-diameter metal disk placed in the abdominal wall. A quadripolar catheter electrode (6F, United States Catheter Instruments, Billerica, Mass.) was advanced from the right carotid artery to the noncoronary cusp of the aorta to record His' bundle activation with frequency responses of 30–500 Hz. Bipolar plunge electrodes in the right atrium and left ventricle were used to record atrial and ventricular responses, respectively. Surface electrocardiographic lead II, His’ bundle electrogram, right atrial and left ventricular electrograms, and arterial blood pressure were recorded on an Electronics for Medicine recorder (Pleasantville, N.Y.) at a paper speed of 100 mm/sec. A storage oscilloscope was also used to monitor changes in sinus cycle length. Data were recorded beginning about 30 minutes after the placement of the plunge electrodes.

Measurements of Effective Refractory Periods

Effective refractory period was determined at each test site by the extrastimulus technique employing a Krannert Medical Engineering programmable stimulator and an isolated constant current source. Each test site was driven with a 2-msec rectangular unipolar stimulus at twice diastolic threshold. Late diastolic thresholds of test sites were measured during each intervention. A train of 9 stimuli (S1) at a constant cycle length of 290–300 msec was followed by a later premature stimulus (S2) that produced a propagated atrial or ventricular response. The atrial or ventricular response to S2 was recorded in lead II, was obtained from the bipolar plunge electrode in the right atrium or left ventricle, and was displayed on a storage oscilloscope. The S1–S2 interval was shortened in steps of 2 msec until S2 failed to produce a propagated response. Twenty seconds later, the S1–S2 interval was increased by 5 msec, and the S1–S2 interval was shortened by 1-msec decrements until S2 failed to produce a propagated response. Repeated S1–S2-interval determinations yielded values within 2 msec of each other or the data were discarded. The effective refractory period was defined as the longest S1–S2 interval at which S2 failed to produce a propagated response.

Measurements of Spontaneous Sinus Cycle Length and AH Interval

Surface electrocardiographic lead II, right atrial, and His bundle electrograms were recorded at a paper speed of 100 mm/sec. Five consecutive cycle lengths were measured and averaged to obtain the sinus cycle length. Although the pacemaker site shifted (defined by a change in activation sequence of the atrial electrograms) during some interventions described below in some dogs, the data are included for analyses.

After obtaining the spontaneous sinus-cycle length, the right atrium was paced at constant pacing cycle lengths of 290–300 msec. The AH interval was measured from the earliest onset of rapid right atrial activity recorded in the His bundle electrogram to the onset of the His potential. The HV interval was measured from the onset of the His bundle deflection to the beginning of ventricular depolarization recorded in the
His bundle lead. Five consecutive AH and HV intervals at a constant atrial pacing cycle length were measured and averaged to obtain the AH and HV intervals, respectively.

Incremental right atrial pacing was carried out to determine the shortest atrial-paced cycle length at which 1:1 atrioventricular conduction occurred. Pacing cycle length was shortened in steps of 10 msec every 5 seconds.

**Experiment Protocol**

**Ansae subclaviae stimulation.** Stimulation of the right and left ansae subclaviae was carried out with separate isolated constant current sources driven by a Grass S88 stimulator. Stimuli were 4-msec rectangular pulses at 3 mA. Frequencies were 1, 2, and 4 Hz.

**Vagal stimulation.** Stimulation of the right and left vagal nerves was performed with separate isolated constant current sources driven by a Kranz Medical Engineering digital stimulator. The current strength was set at 0.05 mA greater than that required to produce asystole with right vagal stimulation and asystole or complete AV block with left vagal stimulation using 4-msec rectangular pulses at 20 Hz. Pulse width and frequency of vagal stimulation were increased progressively to obtain a constant sinus cycle length for experiment Protocols 1-3 or a constant AH interval for Protocol 4 (see below and Table 1). Between interventions, vagal nerves were stimulated to be certain that the sinus and AV nodal responses remained constant.

**Protocol 1.** The objective was to determine the effects of three different levels of simultaneous bilateral ansae subclaviae and vagal stimulation that maintained a constant sinus cycle length. Baseline values were determined first. Five minutes later, bilateral ansae subclaviae were stimulated at 1, 2, or 4 Hz. Two minutes later, the vagi were stimulated bilaterally, adjusting both pulse width and stimulation frequency simultaneously for both right and left vagal nerves to keep sinus cycle length the same as the baseline value. Effective refractory period and AV nodal conduction time were measured 1 minute after the sinus cycle length became stable. Fifteen minutes after terminating all neural stimulation, baseline values were measured again. Then, another combination of ansae subclaviae-vagal stimulation was started, and refractory periods and AV nodal conduction were determined as above. The frequency of ansae subclaviae stimulation was selected in random order, and baseline values were measured both before and after each combination of ansae subclaviae-vagal stimulation.

**Protocol 2.** The objective was to determine the effects of simultaneous unilateral and bilateral ansae subclaviae and vagal stimulation that maintained a constant sinus cycle length. Five minutes after recording the baseline values, stimulation of unilateral ansa subclavia at 2 Hz was started. Two minutes later, the ipsilateral vagus was stimulated, and pulse width and frequency were titrated to keep sinus cycle length the same as the baseline value, as in Protocol 1. Fifteen minutes after terminating all neural stimulation, baseline values were measured again. Then the study was repeated, stimulating both the opposite ansa subclavia at 2 Hz and vagus. The effects of bilateral simultaneous stimulation of ansae subclaviae at 2 Hz and vagi were also determined as in Protocol 1.

**Protocol 3.** Our objective was to determine the effects of stimulation of bilateral ansae subclaviae alone, stimulation of bilateral vagi alone at an intensity required to keep the sinus cycle length constant during ansae subclaviae stimulation and simultaneous stimulation of bilateral ansae subclaviae and vagi. Five minutes after recording baseline values, stimulation of bilateral ansae subclaviae at 2 Hz was started, and parameters were measured. Then, bilateral vagi were stimulated simultaneously at a pulse width and frequency of vagal stimulation titrated to keep the sinus cycle length constant as in Protocol 1, and the parameters were remeasured. After baseline values were determined again, bilateral vagi were stimulated at the same pulse width and frequency as above, and measurements were obtained. Then, stimulation of bilateral ansae subclaviae at 2 Hz was added, and parameters were remeasured. Finally, baseline values were deter-

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**Table 1. Study Protocols**

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Parameter kept constant</th>
<th>Ansae subclaviae stimulation*</th>
<th>Vagal stimulation†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Spontaneous sinus cycle length</td>
<td>Bilateral at 1 Hz</td>
<td>Bilateral</td>
</tr>
<tr>
<td></td>
<td>Spontaneous sinus cycle length</td>
<td>Bilateral at 2 Hz</td>
<td>Bilateral</td>
</tr>
<tr>
<td></td>
<td>Spontaneous sinus cycle length</td>
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<td>Bilateral</td>
</tr>
<tr>
<td>2</td>
<td>None</td>
<td>Right at 2 Hz</td>
<td>Right</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>Left at 2 Hz</td>
<td>Left</td>
</tr>
<tr>
<td></td>
<td>None</td>
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<td>Bilateral</td>
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<td>3</td>
<td>None</td>
<td>Bilateral at 2 Hz</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>Bilateral</td>
<td>Bilateral</td>
</tr>
<tr>
<td>4</td>
<td>Spontaneous sinus cycle length</td>
<td>Bilateral at 2 Hz</td>
<td>Bilateral</td>
</tr>
</tbody>
</table>

*Pulse width, 4 msec; current strength, 3 mA.  
†Current strength was set at 0.05 mA greater than that required to induce asystole or complete AV block using 4-msec pulses delivered at 20 Hz. Pulse width and frequency were then titrated.
Bilateral Ansae Subclaviae and Vagal Stimulation
Effects of Three Different Levels of Simultaneous Bilateral Stimulation

Protocol 1. Our objective was to determine the effects of simultaneous bilateral ansae subclaviae and vagal stimulation that maintained AH interval constant at an atrial pacing cycle length of 300 msec. After obtaining baseline values, the His' bundle electrogram was displayed on a storage oscilloscope at a sweep speed of 20 msec/cm, and each cardiac cycle was superimposed by triggering from the atrial pacing spike. Two minutes after stimulation of bilateral ansae subclaviae at 2 Hz, bilateral vagi were stimulated, and pulse width and frequency were titrated to return the AH interval to within ±4 msec of the baseline value. Then, atrial pacing was terminated. Bilateral vagal and ansae subclaviae stimulation was continued at the same parameters, and spontaneous sinus cycle length and effective refractory periods were measured. The cycle length at which the effective refractory periods were determined was 300 msec.

Data Analysis
Parameters obtained during ansae subclaviae-vagal stimulation were compared with the mean value of two baseline values just prior to and following the particular combination of ansae subclaviae-vagal stimulation, except for Protocol 4. For Protocol 4, values during control state and during ansae subclaviae-vagal stimulation were compared. Changes in high and low cycle length did not differ from that prior to measurement of refractory periods (406 ± 59 vs. 404 ± 57 msec), confirming the stability of the sinus cycle length and its response to neural stimulation during the experiment.

Results
Effects of Three Different Levels of Simultaneous Bilateral Ansae Subclaviae and Vagal Stimulation That Maintained a Constant Sinus Cycle Length (Protocol 1)
Changes in electrophysiologic parameters at three different levels of bilateral ansae subclaviae-vagal stimulation were determined in 10 dogs. Current strength was 0.74 ± 0.32 mA for right vagal stimulation and 0.84 ± 0.63 mA for left vagal stimulation. Other parameters of vagal stimulation are summarized in Table 2. Sinus cycle length did not change by experiment design (Table 2), although shift of pacemaker site was observed in 3 of 10 dogs during ansae subclaviae stimulation at 2 Hz with concomitant vagal stimulation. Baseline AH interval was 87 ± 28 msec and changed variably from dog to dog. AH interval lengthened by 20 msec or more in 3 dogs during simultaneous stimulation of the vagi and ansae subclaviae at 1 and 4 Hz and in 2 dogs during stimulation of ansae subclaviae at 2 Hz (Figure 1). AH intervals shortened by 20 msec or more in 1, 3, and 2 dogs during stimulation of vagi and ansae subclaviae at 1, 2, and 4 Hz, respectively (Figure 1). HV intervals remained unchanged. Changes in refractory period at high right atrium in 8 dogs and at low right atrium in 10 dogs were pooled. Baseline effective refractory periods at right atrium, right ventricle, anterior left ventricle, and posterior left ventricle were 141 ± 15, 154 ± 14, 158 ± 10, and 158 ± 12 msec, respectively. The amount of shortening of effective refractory period increased progressively as the frequency of ansae subclaviae stimulation increased (Figure 2).

Spontaneous sinus cycle length was redetermined in 8 dogs (mean number of stimulation combinations, 2.3 per dog) after measurement of refractory periods at all test sites while continuing neural stimulation. Sinus cycle length did not differ from that prior to measurement of refractory periods (406 ± 59 vs. 404 ± 57 msec), confirming the stability of the sinus cycle length and its response to neural stimulation during the experiment.

Effects of Simultaneous Unilateral and Bilateral Ansae Subclaviae and Vagal Stimulation (Protocol 2)
Changes in parameters induced by stimulation of unilateral and bilateral ansae subclaviae at 2 Hz and vagi to maintain constant sinus cycle length were determined in 9 dogs (Table 3 and Figures 3–4). Current strength was 1.11 ± 0.36 mA for right vagal stimulation and 0.88 ± 0.67 mA for left vagal stimulation. In 6 of 9 dogs, Protocol 1 was also tested. Sinus cycle length remained unchanged by design (Table 3), although shift of pacemaker site was observed in 2 dogs during stimulation of right ansa subclavia and vagus

Table 2. Changes in Sinus Cycle Length and Parameters of Vagal Stimulation for Protocol 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>No stimulation</th>
<th>1 Hz</th>
<th>2 Hz</th>
<th>4 Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency of ansae subclaviae stimulation</td>
<td>2 Hz</td>
<td>4 Hz</td>
<td>4 Hz</td>
<td>4 Hz</td>
</tr>
<tr>
<td>Vagal stimulation pulse width (ms)</td>
<td>…</td>
<td>0.61 ± 0.86</td>
<td>0.83 ± 1.14</td>
<td>1.58 ± 1.36</td>
</tr>
<tr>
<td>Vagal stimulation frequency (Hz)</td>
<td>…</td>
<td>3.0 ± 1.8</td>
<td>3.5 ± 1.6</td>
<td>4.0 ± 1.6</td>
</tr>
<tr>
<td>Sinus cycle length (ms)</td>
<td>420 ± 56*</td>
<td>422 ± 57</td>
<td>415 ± 58</td>
<td>415 ± 56</td>
</tr>
<tr>
<td>Change in sinus cycle length (ms)</td>
<td>0</td>
<td>1 ± 13†</td>
<td>−7 ± 13†</td>
<td>0 ± 12†</td>
</tr>
</tbody>
</table>

*Mean of 4 determinations.
†Changes from mean of two baseline values just prior to and following the particular combination of ansae subclaviae-vagal stimulation.
FIGURE 1. Changes in AH interval in each dog are shown on ordinate as function of frequency of ansae subclaviae stimulation shown on the abscissa. Frequency of 0 Hz indicates baseline state. In 1 dog, Wenckebach periodicity (W) developed during ansae subclaviae stimulation at 4 Hz with concomitant vagal stimulation. Dotted line, baseline level.

FIGURE 2. Changes in effective refractory period of right atrium (RA), right ventricle (RV), anterior left ventricle (LVA), and posterior left ventricle (LVP) are shown on ordinate as function of frequency of ansae subclaviae stimulation shown on the abscissa. Frequency of 0 Hz indicates baseline state. Dotted line, baseline level.

and in another dog during stimulation of left ansa subclavia and vagus. Baseline AH interval was 79 ± 12 msec and was lengthened by 15 msec or more in 2 dogs during stimulation of right ansa subclavia and vagus and in response to bilateral ansae subclaviae and vagi (Figure 3). During stimulation of left ansa subclavia and vagus, AH intervals shortened in all dogs by 5–25 msec. Bilateral ansae subclaviae–vagal stimulation shortened AH intervals by 20 msec or more in 2 dogs. HV intervals remained unchanged in all dogs.

Changes in refractory period at high right atrium in 6 dogs and at low right atrium in 9 dogs were pooled. Baseline effective refractory periods at right atrium, right ventricle, anterior left ventricle, and posterior left ventricle were 138 ± 10, 151 ± 14, 158 ± 11, and 158 ± 11 msec, respectively. Effective refractory period of the right atrium was shortened significantly by stimulation of right ansa subclavia and vagus and by bilateral stimulation but not by stimulation of the left ansa subclavia and vagus (Figure 4). The right ventricular refractory period was not shortened by stimulation of unilateral ansa subclavia combined with ipsilateral vagal stimulation but was shortened significantly by stimulation of bilateral ansae subclaviae and vagi. The refractory period of the anterior left ventricle shortened significantly during each combination of stimulation and shortened more during bilateral stimulation than during left ansa subclavia–vagal stimulation (p < 0.005). The refractory period of the posterior left ventricle shortened significantly during stimulation of left and bilateral ansae subclaviae and vagi but not during right ansa subclavia–vagal stimulation. Sinus cycle length determined after measurement of refractory periods but during unilateral neural stimulation in 7 dogs (mean number of stimulation combinations, 1.6 per dog) did not differ from that obtained before refractory period measurements (409 ± 53 vs. 413 ± 46 msec).

Effects of Stimulation of Bilateral Ansae Subclaviae Alone, Bilateral Vagi Alone at Intensity Required to Keep Sinus Cycle Length Constant During Stimulation of Ansae Subclaviae, and Simultaneous Bilateral Ansae Subclaviae and Vagi (Protocol 3)

In 8 dogs, changes induced by stimulation of bilateral ansae subclaviae at 2 Hz and of bilateral vagi alone at an intensity required to keep the sinus cycle length constant during ansae subclaviae stimulation and of both nerves together were determined. Vagal nerves were stimulated using pulses of 0.71 ± 0.16 msec duration and of 3.3 ± 1.0 Hz. Current strength was 0.84 ± 0.48 mA for the right vagus and 0.41 ± 0.12 mA for the left vagus. Shift of pacemaker site was observed in 3, 4, and 4 dogs during ansae subclaviae stimulation.
Table 3. Changes in Sinus Cycle Length and Parameters of Vagal Stimulation for Protocol 2

<table>
<thead>
<tr>
<th>Site of ansae subclaviae stimulation at 2 Hz</th>
<th>No stimulation</th>
<th>Right</th>
<th>Left</th>
<th>Right and left</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vagal stimulation pulse width (ms)</td>
<td>0.83±0.43</td>
<td>0.42±0.42</td>
<td>0.63±0.42</td>
<td></td>
</tr>
<tr>
<td>Vagal stimulation frequency (Hz)</td>
<td>4.0±2.0</td>
<td>2.7±0.9</td>
<td>3.3±1.6</td>
<td></td>
</tr>
<tr>
<td>Sinus cycle length (ms)</td>
<td>409±48*</td>
<td>411±50</td>
<td>417±50</td>
<td>403±46</td>
</tr>
<tr>
<td>Change in sinus cycle length (ms)</td>
<td>0</td>
<td>4±13†</td>
<td>4±9†</td>
<td>−2±14†</td>
</tr>
</tbody>
</table>

*Mean of 4 determinations.
†Changes from mean of two baseline values just prior to and following the particular combination of ansae subclaviae-vagal stimulation.

Changes in refractory period at high right atrium in 5 dogs and at low right atrium in 8 dogs were pooled. Baseline refractory periods of right atrium, right ventricle, anterior left ventricle, and posterior left ventricle were 139 ± 7, 150 ± 7, 156 ± 9, and 158 ± 10 msec, respectively. Right atrial refractory period was shortened significantly by ansae subclaviae stimulation alone and vagal stimulation alone and was further shortened by simultaneous stimulation of both nerves (Figure 7). Right and left ventricular refractory periods were shortened by stimulation of ansae subclaviae alone and by simultaneous stimulation of both nerves. Bilateral vagal stimulation, both alone and when added to bilateral ansae subclaviae stimulation, tended to lengthen refractory periods of right and left ventricles, but the difference was not significant. Sinus cycle length determined after refractory period measurement but during stimulation of both nerves in 4 dogs (mean number of stimulation combinations, 1.8 per dog) did not differ from that prior to refractory period measurements (449 ± 19 vs. 454 ± 20 msec).

Effects of Simultaneous Bilateral Ansae Subclaviae and Vagal Stimulation That Maintained AH Interval Constant at a Fixed Atrial Pacing Cycle Length (Protocol 4)

In 7 dogs, AH intervals at an atrial pacing cycle length of 300 msec were kept constant during bilateral ansae subclaviae-vagal stimulation. In 4 of these 7 dogs, Protocol 3 also was tested. Vagal stimulation was carried out using pulses of 0.81 ± 0.25 msec duration at 3.0 ± 1.0 Hz. Current strength was 0.87 ± 0.57 mA for the right vagus and 0.35 ± 0.08 mA for the left vagus. Changes in AH interval and refractory periods are summarized in Table 4. After cessation of atrial pacing, shift of pacemaker site occurred in 1 of 7 dogs. Sinus cycle length lengthened in 4 dogs by 15, 90, 90, and 95 msec and shortened in 3 dogs by 10, 15, and 15 msec. Refractory period at each test site shortened significantly during ansae subclaviae-vagal stimulation (Table 4).

Discussion

The major finding of the present study is that, while the spontaneous sinus cycle length was kept constant by titrating levels of vagal stimulation against various combinations and strengths of sympathetic stimulation, effective refractory periods of the right atrium, right ventricle, and left ventricle shortened progres-
sively as the frequency of ansae subclavia stimulation increased. AV nodal conduction either remained the same, shortened, or delayed depending on the neural combinations used. When vagal stimulation was titrated against the effects of ansae subclavia stimulation to maintain constant AV nodal conduction time, atrial and ventricular refractoriness still shortened and the spontaneous sinus cycle length lengthened in 4 dogs and shortened in 3 dogs.

Many of these responses could have been predicted, given the known dominance of efferent vagal effects on sinus nodal automaticity, the opposite but algebraically additive effects of efferent vagal and sympathetic stimulation on AV nodal conduction, the synergistic effects of efferent vagal and sympathetic stimulation on atrial refractoriness, and the opposing effects on ventricular refractoriness with sympathetic effects potent, and the general “sidedness” of right and left efferent vagal and sympathetic distributions. However, despite many studies investigating the effects of vagal–sympathetic interactions on a variety of electrophysiologic properties, to the authors’ knowledge there have been no studies reported in which the spontaneous sinus cycle length has been kept constant.

One reason for our study was to investigate the use of the spontaneous sinus nodal cycle length as a kind of autonomic barometer for the rest of the heart. Our data provide experiment support for the conclusion that the sinus cycle length may not be an accurate indicator of autonomic tone to other cardiac structures under all circumstances, most likely when discharge of both autonomic limbs occurs simultaneously. Given the possibility that simultaneous discharge of both au-
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Inoue and Zipes  Conduction and ERP Changes at a Constant SCL

Changes in Sinus Node Automaticity and Atrial Refractory Period

The inhibitory effects of a given level of vagal activity on heart rate become more pronounced when the prevailing level of sympathetic activity increases (accentuated antagonism). Since both pulse width and frequency of vagal stimulation were changed in the present study, accentuated antagonism of the heart rate between vagal and sympathetic nerves was not produced.

While it is well established that vagal stimulation shortens atrial refractoriness in the canine heart, the effects of sympathetic stimulation are inconsistent possibly because stimulation of α-receptors lengthens atrial refractory periods while stimulation of β-receptors shortens it. In the present study, bilateral ansae subclaviae stimulation alone shortened the refractory period of the right atrium significantly and shortened it progressively as the frequencies of ansae subclaviae stimulation and of vagal stimulation increased. Stimulation of the right ansa subclavia and vagus shortened the refractory period of the right atrium, but stimulation of left ansa subclavia and vagus did not, which is consistent with previous studies.

Changes in Ventricular Refractory Period

In the present study, ansae subclaviae stimulation alone shortened the refractory period of the right and

![Figure 7](image-url) Changes in effective refractory period of right atrium, right ventricle, anterior left ventricle, and posterior left ventricle are shown. Dotted line, baseline level; p values compared with the control value are shown unless otherwise specified.

![Figure 8](image-url) Change from control of shortest pacing cycle length (PCL) with 1:1 AV conduction (ordinate) is plotted as function of change from control of AH interval. Closed symbols, bilateral ansae subclaviae stimulation at 1, 2, and 4 Hz with concomitant vagal stimulation; open symbols, unilateral ansae subclaviae stimulation at 2 Hz with concomitant vagal stimulation. Parameters of vagal stimulation are not shown in figure. Significant correlation occurred between changes in AH interval and shortest PCL with 1:1 AV conduction.

Table 4. Changes in Parameters for Protocol 4 After Ansae Subclaviae and Vagal Stimulation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>Ansae subclaviae and vagal stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>AH interval (ms)</td>
<td>114 ± 15</td>
<td>115 ± 15</td>
</tr>
<tr>
<td>Effective refractory period (ms)</td>
<td>145 ± 10</td>
<td>123 ± 9*</td>
</tr>
<tr>
<td>RA</td>
<td>155 ± 12</td>
<td>147 ± 10†</td>
</tr>
<tr>
<td>RV</td>
<td>162 ± 12</td>
<td>146 ± 7‡</td>
</tr>
<tr>
<td>LVa</td>
<td>163 ± 11</td>
<td>148 ± 13§</td>
</tr>
<tr>
<td>LVp</td>
<td>123 ± 9*</td>
<td>123 ± 9*</td>
</tr>
</tbody>
</table>

RA, right atrium; RV, right ventricle; LVa, anterior left ventricle; LVp, posterior left ventricle.

*p < 0.001, †p < 0.01, §p < 0.02.
left ventricles significantly. Vagal stimulation alone tended to lengthen the ventricular refractory period, possibly because of the presence of heightened sympathetic tone in anesthetized, open-chest dogs. Adding vagal stimulation to ansae subclaviae stimulation also tended to lengthen the ventricular refractory period, but the difference was not significant, possibly due to the relatively short pulse width and low frequency of vagal stimulation. Thus, despite antagonism of sympathetic effects by the vagus at both prejunctional and postjunctional levels and possible direct vagal effects, sympathetic activity predominated in control of ventricular refractoriness. Thus, during simultaneous stimulation of ansae subclaviae and vagi, the effective refractory periods of the right and left ventricles shortened progressively as the frequency of ansae subclaviae stimulation increased.

As expected, stimulation of the left ansa subclavia and vagus and bilateral ansae subclaviae and vagi but not stimulation of right ansa subclavia and vagus shortened the refractory period of the posterior left ventricle. Stimulation of right or left ansa subclavia and vagus shortened the refractory period of the anterior left ventricle, more so during bilateral stimulation than during stimulation of left ansa subclavia and vagus. Haws and Burgess showed that left stellate stimulation shortened refractory periods to the same extent as bilateral stellate stimulation at ventricular sites, which received overlapping innervation from the right and left stellate ganglia. The difference between their results and ours may be explained by predominant innervation of the right sympathetic nerves to the test site of the present study and/or by lower frequency of ansae subclaviae stimulation in the present study, which did not elicit a maximal sympathetic response.

Atrioventricular Nodal Conduction

Spear et al. showed that the magnitude of shortening in AV nodal conduction induced by stellate stimulation was smaller than the lengthening produced by vagal stimulation. Simultaneous stimulation of the stellate ganglia and the vagi elicited algebraically additive effects on AV nodal conduction in contrast to accentuated antagonism observed in control of sinus rate. Under constant background sympathetic tone, AH interval lengthened as the frequency of vagal stimulation increased. In the present study, during stimulation of bilateral ansae subclaviae and vagi, which kept sinus cycle length constant, changes in AH intervals varied in different dogs. This might be explained by different AV nodal distributions of the vagus and sympathetics and/or by different sensitivity between sinus node and AV node to vagal activity and sympathetic activity. In some dogs, the effects of right vagal stimulation on AV nodal conduction predominated over that of right ansae subclaviae stimulation, while in other dogs, the effect of right ansae subclaviae stimulation predominated. Left ansae subclaviae stimulation shortened AV nodal conduction despite left vagal stimulation in each instance.

When we performed the inverse experiment, which kept the AH interval constant and measured the spontaneous sinus cycle length, we found that atrial and ventricular refractoriness shortened in each dog, while spontaneous sinus cycle length lengthened in 4 of 7 dogs.

Methodological Consideration

Tonic discharge of sympathetic cardiac nerves was reported to be at 1–3 Hz. In the present study, the frequency of ansae subclaviae stimulation was selected at 1–4 Hz to minimize changes induced by supramaximal stimulation used in some previous studies. Shift of pacemaker site was observed during neural stimulation in some dogs. These dogs were included in the analysis, since priority was given to creating a constant basic cycle length. In these dogs, the P wave was still positive in surface lead II, suggesting that the new pacemaker site still may have been in the high right atrium.

Implications

The present data show that spontaneous sinus cycle length is not necessarily an autonomic barometer of neural input to the AV node, atrium, and ventricle of the heart. In a previous study, Browne et al. showed that Q–T interval in humans was significantly longer during sleep than during the awake state at the same heart rate. A supporting observation was made by Bexton et al. Browne et al. speculated that the same sinus rate at different times did not necessarily mean the same degree of autonomic input to the ventricle, which explains the change in Q–T interval. The present data support their speculation. Thus, the same spontaneous sinus rate does not necessarily mean that the autonomic input to the rest of the heart is the same. Also, situations that may be characterized by increased discharge from both autonomic limbs, e.g., acute inferior myocardial infarction with a vasodepressor reflex response, may show electrocardiographic evidence of vagal effects at the sinus and, possibly, AV nodes, which may mask intense adrenergic effects in the ventricle. Changing our stimulation parameters, e.g., increasing the strength of vagal stimulation, just as easily could have resulted in sinus slowing and possibly in prolongation of AV nodal conduction time while retaining shortening of atrial and ventricular refractoriness as long as concomitant ansae subclaviae stimulation resulted. Were these sinus and AV nodal changes to happen clinically, it would have been concluded almost certainly that the patient was experiencing a "vagotonic state" without considering the possibility of simultaneous sympathetic discharge and its effects on atrial and ventricular refractoriness or other properties.

Acknowledgment

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References

1. Levy MN, Martin PJ: Neural control of the heart, in Berne RM, Sperelakis N (eds): Handbook of Physiology: Circulation, Sec-
Conduction and ERP Changes at a Constant SCL

21. Thames MD, Klopfenstein HS, Abboud FM, Mark AL, Walke-
er JL: Preferential distribution of inhibitory cardiac receptors
with vagal afferents to the inferoposterior wall of the left
ventricle activated during coronary occlusion in the dog. Circ Res
1978;43:512–519

22. Wallenstein S, Zucker CL, Fleiss JL: Some statistical methods

23. Alessi R, Nusynowitz M, Abildskov JA, Moc GK: Nonuni-
form distribution of vagal effects on the atrial refractory peri-

24. Govier WC, Mosal NC, Whintning P, Broom AH: Myocardial-
alpha and beta adrenergic receptors as demonstrated by atrial
functional refractory period changes. J Pharmacol Exp Ther
1966;154:255–263

25. Pappano AJ: Propranolol-insensitive effects on action potential
repolarization in electrically driven atria of the guinea pig. J
Pharmacol Exp Ther 1971;177:85–95

26. Randall WC: Selective autonomic innervation of the heart, in
Randall WC (ed): Nervous Control of Cardiovascular Func-

27. De Elio FJ: The action of acetylcholine, adrenaline and other
substances on the refractory period of the rabbit auricle. Br J
Pharmacol 1947;21:131–142

28. Di Palma JR, Mascatello AV: Analysis of the action of acetyl-
choline, atropine, epinephrine, and quinidine on heart muscle

29. Giotti A, Ledda F, Mannioni PF: Effects of noradrenaline and
isoprenaline in combination with alpha and beta-receptor
blocking substances on the action potential of cardiac Purkinje

30. Blair RW, Shimizu T, Bishop VS: The role of vagal afferents
in the reflex control of the left ventricular refractory period in

31. Prystowsky EN,Jackman WM, Rinkenberger RL, Heger JI,
Zipes DP: Effect of autonomic blockade on ventricular refrac-
toriness and atrioventricular nodal conduction in humans: Evi-
dence supporting a direct cholinergic action on ventricular

32. Haws CW, Burgess MJ: Effects of bilateral and unilateral
stellate stimulation on canine ventricular refractory periods at

33. Spear JF, Moore EN: Influence of brief vagal and stellate nerve
stimulation on pacemaker activity and conduction within the
atrioventricular conduction system of the dog. Circ Res
1973;32:27–41

34. Urrthaler F, Neely BH, Hageman GR, Smith LR: Differential
sympathetic-parasympathetic interactions in sinus node and

35. Folkow B: Impulse frequency in sympathetic vasorriotor fibers
correlated to the release and elimination of the transmitter.
Acta Physiol Scand 1952;25:49–76

36. Vassalle M, Levine MJ, Stuckey JH: On the sympathetic con-
trol of ventricular automaticity: The effects of stellate ganglion

37. Browne KD, Prystowsky E, Heger JJ, Chilson DA, Zipes DP:
Prolongation of the Q-T interval in man during sleep. Am J
Cardiol 1983:52:53–59

38. Bexton RS, Vallin HO, Camm AJ: Diurnal variation of the QT
interval-influence of the autonomic nervous system. Br Heart J
1986;55:253–258

KEY WORDS • vagus • sympathetics • sinus-cycle length
• refractoriness • atrioventricular conduction
Changes in atrial and ventricular refractoriness and in atrioventricular nodal conduction produced by combinations of vagal and sympathetic stimulation that result in a constant spontaneous sinus cycle length.

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