Direct Evidence of Nonuniform Distribution of Vagal Effects on Dog Atria

By Ishio Ninomiya, M.D.

ABSTRACT

The duration of the monophasic action potential (APD), the interval between atrial beats and the delay time between excitation of the right and left atria of dogs were measured, using coaxial suction electrodes, before and during vagal stimulation.

The effect of vagal stimulation on the degree of shortening of APD varied at different recording sites in the atrium, the shortening being more pronounced in the right than in the left atrium. Threshold and rate of recovery after cessation of stimulation varied throughout the atrium. The results indicate that the vagal effect is nonuniformly distributed over the atrium.

The time difference between excitation of the right and left atria increased with vagal stimulation under the present experimental condition, suggesting that a reduction of synchrony of excitation occurred in the atrial myocardial fibers as a whole. The same conclusion was derived from analysis of the pattern of the P wave before and during vagal stimulation.

ADDITIONAL KEY WORDS

vagal stimulation, monophasic atrial action potential, averaged P wave, shift of pacemaker, atrial fibrillation, synchronization of atrial excitation, coaxial suction electrode, data-averaging computer, right vs. left atrium

Studies on the distribution of vagal effects in the atrium provide important data regarding the frequency, conduction and recovery of myocardial excitation in normal and pathologic conditions such as tachycardia, flutter, and fibrillation. Measurement of the refractory period in various parts of the right atrium in the dog during vagal stimulation has shown that the vagal effect is nonuniformly distributed. Such nonuniformity of vagal effects is assumed to be one of the possible causes of atrial fibrillation.

Recently, the effect of vagal stimulation or acetylcholine on electrically driven or spontaneously active atrial fibers has been studied with intracellular electrodes and also with monophasic records taken from the turtle atrium. These studies have demonstrated that the duration of the action potential is shortened dramatically during vagal stimulation. The duration of the monophasic action potential (APD) can be used as an index of the vagal effect on the myocardial fibers in various parts of the atrium. The purpose of the current experiments is to secure information on the distribution of vagal effect in the canine heart in situ.

METHODS

Ten mongrel dogs (6 to 10 kg) were used in this study. Each was anesthetized with sodium pentobarbital (Nembutal, 25 mg/kg), the trachea was intubated and the lungs were ventilated with intermittent positive pressure from a Harvard pump. Additional doses of 5 mg/kg sodium pentobarbital were injected intravenously whenever the electromyogram confounded the electrocardiogram (ECC). The heart was exposed by a transsternal approach at the third intercostal space. Two coaxial suction electrodes were used in recording the monophasic potentials simultaneously from both sides of the atria.
The details of the coaxial suction electrodes have been described previously. Since the coaxial suction electrode is flexible enough to follow the vigorous movement of the atrial surface, monophasic potentials could be recorded for a long time after applying negative pressure with a hydroaspirator. The changes in monophasic action potential detected by the recording electrodes were fed into a conventional high-grain differential d-c amplifier and displayed on a four-channel cathode ray oscilloscope (Model VC 7, Nihon Koden). Since it is difficult to determine the end of repolarization precisely, in the present experiments, APD was measured as the interval between the onset of depolarization and the intercepted time obtained by the extrapolation of the repolarization slope to the base line.

The vagus nerves were cut high in the neck. Either the right or left peripheral trunk was placed on bipolar silver electrodes and stimulated with square-wave shocks of 5 msec in pulse width and 20 cycle/sec in frequency by an electronic stimulator (Model 160 series, Tektronix). The intensity of the stimulation was varied from 2 to 16 volts. Lead II ECG was recorded simultaneously to show the pattern of A-V conduction, to analyze the effect of vagal stimulation on the configuration of the P wave, and to monitor the electromyogram. The averaged P-wave pattern was obtained by a digital memory averaging device (ND 800, Enhancetron, Nuclear Data, Inc.). During the experiment, warm physiologic saline solution was dropped on the epicardium to maintain constant moisture and temperature. The temperature of the atrial surface was approximately 32°C and room temperature was 25°C.

## Results

### EFFECT OF VAGAL STIMULATION ON APD

Vagal stimulation of low intensity primarily affects the frequency of pacemaker activity. Vagal stimulation of relatively high intensity was needed to significantly alter the atrial APD in all sites at which it was measured. For example, during right vagal stimulation, the APD in the right atrium shortened slightly from 215 ± 12 msec to 186 ± 11 msec, i.e., to 86% of the control value, while the interval between atrial beats increased remarkably from 452 ± 9 msec to 1249 ± 305 msec, i.e., to 276% of the control value (Table 1). At the onset of vagal stimulation, there was a rapid change in the plateau phase from upward concavity to downward concavity in the monophasic action potential pattern, and

<table>
<thead>
<tr>
<th>Effect of Vagal Stimulation</th>
<th>L. atrium</th>
<th>R. atrium</th>
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<tbody>
<tr>
<td>Control</td>
<td>276 ± 9</td>
<td>237 ± 9</td>
</tr>
<tr>
<td>1</td>
<td>271 ± 8</td>
<td>238 ± 7</td>
</tr>
<tr>
<td>2</td>
<td>267 ± 10</td>
<td>232 ± 9</td>
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<td>3</td>
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<td>259 ± 14</td>
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<td>215 ± 9</td>
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<tr>
<td>6</td>
<td>251 ± 18</td>
<td>210 ± 9</td>
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<tr>
<td>7</td>
<td>247 ± 20</td>
<td>205 ± 9</td>
</tr>
<tr>
<td>8</td>
<td>243 ± 23</td>
<td>201 ± 9</td>
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<tr>
<td>9</td>
<td>240 ± 25</td>
<td>197 ± 9</td>
</tr>
<tr>
<td>10</td>
<td>237 ± 27</td>
<td>193 ± 9</td>
</tr>
</tbody>
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*Standard deviation.*
Sequential changes in the duration of the monophasic action potential during vagal stimulation (APD). APD was recorded at one site on the right atrium at the onset (A) and end (B) of right vagal stimulation. The 10-msec time calibration in A applies to A and B. Voltage calibration is 10 mv. In C, APD (ordinate) was measured in a series of cardiac cycles (abscissa). Horizontal bar in C shows the interval of vagal stimulation.

Shortening in the APD resulted from shortening of the second phase of the plateau. The data illustrated in Figure 1 were recorded from one site of the right atrial appendage. After the onset of vagal stimulation, the APD shortened to almost minimum length within three cardiac cycles and then continued at a relatively constant level (Fig. 1, A and C).

After cessation of stimulation, the APD quickly recovered to one-half of the control level, and then gradually returned to the previous level (Fig. 1, B and C). These observations almost coincided with the previous results obtained with the ultramicroelectrode technique. In many instances, the time course of recovery in the APD after cessation of vagal stimulation was different from site to site within the same atrial tissue. Two examples are illustrated in Figures 2 and 4.

**FIGURE 2**

Time course of recovery in APD at seven different recording sites after cessation of right vagal stimulation. At zero cardiac cycle the stimulation ceased. Insert is a schematic illustration of recording sites as shown by different symbols.

**DIFFERENCES IN THE THRESHOLD TO VAGAL STIMULATION AT VARIOUS SITES OF THE ATRIUM**

The shortening of the atrial APD could not be observed until the stimulus intensity was increased to a certain voltage. In the example shown in Figure 3, monophasic action potentials were recorded at four different sites on the right atrium before and during vagal stimulation. In the vicinity of the pacemaker region the APD was longest in the control, and shortened at the lowest intensity. At 6 volts it decreased to about 89% below that of the control level, but in other regions (2 and 3 in Fig. 3), the duration shortened only when the stimulus intensity was greater than 6 volts. Figure 3 clearly shows that for a given intensity of vagal stimulation the degree of shortening of the APD varied at different recording sites. These differences in the...
threshold to vagal stimulation and in the degree of shortening of the APD were observed in all other experiments, which indicates that the effect of vagal stimulation is not uniform in various parts of the atrium. A significant increase in the standard deviation of the APD during vagal stimulation in all experiments suggests that there is a remarkable variation of the APD at different recording sites of the atrium during vagal stimulation (Table 1).

COMPARISON OF RIGHT AND LEFT ATRIAL MONOPHASIC ACTION POTENTIALS

Monophasic action potentials were recorded simultaneously from the right and left atrium in ten instances. The mean APD was 215 ± 12 msec in the right and 188 ± 11 in the left atrium, a difference of 15%. During right vagal stimulation, the APD in the right atrium was shortened to 132 ± 16 msec, i.e., to 62% of the control duration, while that in the left atrium decreased to 135 ± 13 msec, i.e., to 73% of the control level (Table 1 and Fig. 4). Thus, right vagal stimulation caused a greater reduction of the APD in the right atrium than in the left atrium. Similar findings were observed during left vagal stimulation.

EFFECT OF VAGAL STIMULATION ON THE SYNCHRONIZATION OF EXCITATION IN THE ATRIA

The delay between the onset of excitation in the right atrium and that in the left was measured in all experiments before and during vagal stimulation by using two electrodes.
Time difference of excitation between the two atria increased from $30 \pm 1.6$ (SE) msec to $35 \pm 1.9$ (SE) msec during right vagal stimulation and to $34 \pm 1.5$ (SE) msec during left vagal stimulation. The difference was significant at the 5% level. In the example illustrated in Figure 4, two monophasic action potentials were recorded simultaneously at both the pacemaker region and the left atrial appendage during right and left vagal stimulation of constant intensity. However, in some cases the delay time of excitation between two different sites in the right atrium shortened slightly. The increase in mean delay time of onset of excitation between the right and left atrium suggests that the net result of vagal stimulation is a reduction in the synchrony of excitation in the atrial myocardium as a whole under the present experimental condition.

CONFIGURATION OF THE P WAVE DURING VAGAL STIMULATION

The amplitude, duration and shape of the P wave of Lead II ECG changed during vagal stimulation, as can be seen in Figure 4. In order to analyze the effect of vagal stimulation on the P-wave pattern more precisely, the averaged P wave was recorded by the method described previously. In Figure 5, two examples of the averaged P-wave pattern during control (1A and 2A) and during vagal stimulation (1B and 2B) are illustrated. In general, as shown previously, two notches were found in the configuration of the averaged P wave in both normal human subjects and dogs. The initial notch (arrow in Fig. 5) was deeper than the second notch, which was often obliterated by noise. In most cases pronounced notches, decrease in amplitude, and prolonged duration of the P wave were observed during vagal stimulation. These phenomena may reflect a change in the summation of individual action potentials in the atrium as a whole, because of a reduction in the synchrony of atrial excitations.

Discussion

In the present study, monophasic action potentials from atrial myocardial fibers before and during vagal stimulation were recorded by using two coaxial suction electrodes. In the attempt to detect differences in vagal effect on the APD, several variables were selected: (1) threshold intensity, (2) degree of shortening in the APD, and (3) time course of recovery from shortening of duration after the cessation of vagal stimulation. From the
measurements of these variables the author has demonstrated a nonuniform distribution of vagal effect in different sites of the atrium, although the monophasic action potentials were recorded from an injured area of approximately 0.6 mm² instead of a single cell.

The results depicted in Figure 3 suggest that all points are susceptible to vagal stimulation, provided only that the nerve is maximally stimulated. This does not mean that the vagal effect on the atrium is uniformly distributed, for as described in the foregoing section, the threshold to vagal stimulation is different in various parts of the atrium. Previous studies on the electrophysiology of the myocardium indicate that there is a limitation in the shortening of the APD, and therefore at maximal stimulus intensity, a nonuniform distribution of the vagal stimulation is difficult to demonstrate. Moreover, as shown in Figure 2, the time course of recovery in the APD after the cessation of vagal stimulation was different from site to site within the same atrial tissue, although the APD shortened maximally. The variation in the threshold to vagal stimulation and in the degree of shortening for a given stimulus intensity at different sites of the atrium was probably due to either the different distribution of vagal terminals or the nonuniformity of sensitivity to acetylcholine released from vagal terminals. In either case, during vagal stimulation there may be areas which are repolarized early, while neighboring areas remain depolarized. This may provide flow of local current from the early repolarized area to the neighboring depolarized area, causing re-excitation of the earlier repolarized area and finally leading to tachycardia, flutter and atrial fibrillation. An example of this is illustrated in Figure 6, A and B. This has been predicted by several investigators. Furthermore, stimulation of the vagal trunk might directly or reflexly activate sympathetic fibers to the heart, and this may oppose the vagal effect. Immediately after the cessation of vagal stimulation, an overshoot increase in the heart rate was noted in all experiments, which suggests an increase in the sympathetic effect on the frequency of excitation (Fig. 6, C). However, such overshoot response could not be observed in the APD. It is well known that the effect on the APD of stimulating the sympathetic nerve is far less than that of the vagus nerve; therefore, in the present study, the sympathetic effect on the APD was not considered as an important factor in causing the nonuniformity of vagal effect.

![Figure 6](https://example.com/figure6.png)

**Figure 6**
Continuous records of Lead II ECG (upper trace) and monophasic action potentials (middle and lower traces) during and after stimulation of the right vagus. In A, at the 5th cardiac cycles the right vagus was stimulated (arrow) and at the third cardiac cycle in B the stimulation has ceased (arrow). Monophasic potentials were recorded near the pacemaker region. Distance between two electrodes was approximately 2 mm in diastole. Tachycardia, flutter, and fibrillation occurred suddenly during vagal stimulation and disappeared after cessation of vagal stimulation. In another record of the same experiment (C), the monophasic action potentials were recorded continuously immediately after cessation of vagal stimulation (arrow). An overshoot increase in heart rate occurred, and then approached the control level. Time calibration, 200 msec. Voltage calibration, 10 mv.
It is well known that one of the characteristics of cardiac action potentials is the variation of repolarization with variations in ionic composition of the bathing medium,12,18 stimulus rate,14 temperature,15 and cell types. The factors causing variation in the APD of the right atrium in the dog heart in situ have been discussed previously.10 In the present experiments, significant differences were noted in the APD between the right and left atrium (Table 1). Such variation in duration may be due partly to the difference in temperature between the atria. Because no attempt was made to control spontaneous frequency of the heart in these studies, the recorded data may represent the result of frequency-dependent and acetylcholine-dependent changes. However, a previous study on the dog atria has shown that the APD does not shorten until the heart rate exceeds 60 to 100 beats/min.16 As seen in Table 1, during vagal stimulation the heart rate exceeded 100 beats/min in only 4 of 20 instances. Furthermore, immediately after cessation of vagal stimulation, the heart rate remarkably increased, but no shortening of the APD was found (Fig. 6, C). These findings suggest that frequency-dependent changes may not be a major factor in shortening the APD during vagal stimulation in this experiment.

Using an artificial pacemaker, Hoffman et al.9 discovered an increase in conduction velocity of excitation in the in situ canine atrium during vagal stimulation, suggesting that there must be an increment in synchronization of excitation. On the other hand, the conduction velocity of excitation has been found to decrease by administering carbamylcholine to a strip of cat atrial myocardium.7 In the present study, the author did not measure the conduction velocity of excitation, but measured the delay time of excitation between multiple sites of the right and left atrium before and during vagal stimulation. This is because the measurement of delay time of excitation between multiple sites can provide im-

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**FIGURE 7**

In A, two parts of continuous records of ECG (upper trace) and monophasic action potential in the right (middle trace) and left atrium (lower trace) are shown. The right vagus was stimulated at the first cardiac cycle in first part (arrow). At the third cardiac cycle, the excitation in both atria occurred almost simultaneously and then the excitation of left atrium preceded that of the right atrium. After the second cardiac cycle in the second part of A when vagal stimulation ceased (arrow), the excitation of left atrium followed that of the right atrium. Such records suggest that the pacemaker region shifted from the right atrium toward the left atrium during vagal stimulation. A negative deflection in configuration of the P wave occurred. In B, monophasic action potentials of another dog were recorded at two different sites on the right atrium. During vagal stimulation the delay time of excitation between two sites randomly changed (first part), but after the cessation of stimulation it remained constant (second part). Such variation in delay time of excitation suggests that there is a shift of pacemaker region from cycle to cycle. Time calibration, 100 msec. Voltage calibration 10 mV. Rising limb of action potentials and QRS spikes were retouched.
important data regarding the excitation pattern of the atrium as a whole. In some cases, the pacemaker region shifted markedly from beat to beat on the atrial surface during vagal stimulation. Figure 7, A, shows that during vagal stimulation the pacemaker region might have shifted toward the left atrium and back again. Another example of this is illustrated in Figure 7, B. Such variation in pacemaker region does not permit a precise determination of conduction velocity of excitation in the spontaneously active atrium during vagal stimulation. However, the mean values of delay time of excitation between two atria was rather increased, although the sites of the electrodes were fixed before and during vagal stimulation. Therefore, there must be a reduction in synchrony of excitation in the atria rather than augmentation. Reduction in synchrony of excitation during vagal stimulation was also suggested from reduced amplitude, increased interval and reinforcement of notches in the P wave (Fig. 5). The increased delay time of excitation between the right and left atrium may be due either to a partial block in the conduction pathway from the right to left atrium or to random distribution of the pacemaker region in the right atrium during vagal stimulation, but not to increase in conduction velocity of excitation as a whole, under the conditions of stimulation used in the present experiments.

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References

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