IN 1845, Weber and Weber were first to demonstrate that vagal stimulation slows the heart. In 1934, Brown and Eccles described for the cat sinus node the time course of changes in spontaneous rhythm following a single vagal volley. Characteristically, the time course of rhythm change exhibits two inhibitory peaks separated by a transient period. Other experiments from several laboratories have provided information about the mechanism of this double-peaked inhibitory time course following vagal stimulation for pacemakers located within the atrioventricular junction (Spear and Moore, 1973).

The Effect of Brief Vagal Stimulation on the Isolated Rabbit Sinus Node

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SUMMARY We developed an isolated rabbit atrial preparation which responds consistently and reproducibly to brief, submaximal stimulation of the autonomic nerves contained in it. In 6 of 11 preparations in the presence of propranolol (1 mg/liter), the time course of changes in the atrial rate following 120 msec vagal stimulation was bimodal. The maximal slowing occurred at 0.84 ± 0.16 second, and the peak secondary slowing occurred at 2.3 ± 1.0 seconds. An acceleratory component occurred between the first and second peaks between 0.8 and 1.8 seconds. The total time course of vagal effect lasted for 5.0 ± 2.0 seconds. These changes in rate could not be explained by shifts in the location of the primary pacemaker. The acceleratory component was due to a 4.7 ± 2.0 (mV) depolarization of the maximum diastolic membrane potential of the primary pacemaker of the sinus node which lasted for 1.8 ± 0.3 seconds. Following vagal stimulation, there was an increase of 0.2 mM in the activity of potassium in the extracellular space recorded with a potassium-sensitive electrode; this peaked between 1.4 and 2.5 seconds and cleared with an exponential time course. The halftimes for recovery ranged between 2.8 and 4.6 seconds. The initial peak slowing of the bimodal time course and the acceleratory component therefore appear to be direct effects of acetylcholine. The secondary slowing occurs after acetylcholine presumably has been inactivated and occurs coincidently with the accumulation of potassium in the extracellular space. Circ Res 44: 75-88, 1979
Potassium was found to accumulate in the extra-cellular space for several seconds after vagal stimulation (Chiba et al., 1975). This indicates that the complex response is not due to the pattern of presynaptic release of acetylcholine. Both of the inhibitory components and the acceleratory component of the response to vagal stimulation are eliminated by atropine (Brown and Eccles, 1934; Levy et al., 1970; Spear and Moore, 1973). Therefore, the mechanism for the complex time course of vagal effect must either reside in the intrinsic response of the sinus node to acetylcholine or be due to acetylcholine-induced release of some secondary mediator.

The original demonstration by Meek and Eyster (1914) that the location of the dominant pacemaker within the sinus node can shift in response to vagal stimulation has been substantiated by other investigators (West, 1955; Bouman et al. 1968). However, Brown and Eccles (1934) concluded that such a shift in pacemaker site could not fully account for the complex time course of rate change following vagal stimulation. Recently, Jalife et al. (1977) demonstrated that a shift in the pacemaker site following vagal stimulation can electrotonically accelerate the primary pacemaker and paradoxically shorten the interval between sinus beats following vagal stimulation. Another recent study (Spear and Moore, 1978) showed that vagally induced reentry within the sinus node may produce a bimodal inhibitory time course.

It is known that vagal stimulation increases potassium efflux from the atrium (Hutter 1961), and this is due to a vagally mediated increase in membrane conductance to potassium (Burgen and Terroux, 1953). Potassium accumulation in the extracellular space may play a role in the rhythm changes following vagal stimulation (Kronhaus et al., 1978).

The present studies demonstrate that the bimodal time course of vagal effects can occur independently of a shift in pacemaker location or reentrant activity. Furthermore, the mechanism for the acceleratory component is shown to involve vagally induced acceleration of the primary pacemaker rate as well as changes in sinoatrial conduction time. Potassium was found to accumulate in the extracellular space for several seconds after vagal stimulation.

Methods

Rabbits weighing between 1 and 3 kg were anesthetized with intravenous sodium pentobarbital (40 mg/kg). The chest was opened and the heart excised along with the superior vena cava and adjoining trachea. The specimen was placed in oxygenated Tyrode’s solution and maintained at 37°C. The composition of the Tyrode’s solution in mm/liter was as follows: NaCl, 137; NaHCO3, 12; dextrose, 5.5; Na2HPO4, 0.9; MgCl2, 0.5; KCl, 2.7; CaCl2, 1.2.

The solution was gassed with 95% oxygen and 5% carbon dioxide. In the experiments, either propranolol (1.0 mg/liter) or atropine (1.0 mg/liter) was added to the Tyrode’s solution. The endocardial surface of the atrium was exposed according to the dissection of Paes de Carvalho et al. (1959). The cut was made along the junction of the right atrial free wall and interatrial septum adjacent to the root of the aorta beginning at the tricuspid valve and extending out of the superior vena cava.

Vagal stimulation was carried out by a previously described (Spear and Moore, 1978) modification of the technique of Vincenzi and West (1963). Close bipolar silver electrodes separated by 1 mm were placed on the endothelial surface of the superior vena cava in the region where vagal fibers innervating the sinus node area course (King and Coakley, 1958). The vagal stimulation was delivered for 120 msec and consisted of 4-msec constant-current rectangular pulses at a frequency of 100 Hz. The intensity of vagal stimulation was submaximal and ranged between 0.1 and 1.0 mA. Maximal stimulation at this duration and frequency in the various preparations produced a maximum increase in the interval between sinus beats ranging between +60 and +220% change above control intervals. In our experiments, the submaximal stimulus intensity was that which produced 50–60% of maximal slowing in a given preparation. The stimuli were synchronized to the cardiac cycle and the time of the cardiac cycle at which they were delivered during the testing sequences was chosen randomly. In these preparations the magnitude of the inhibitory response to vagal stimulation remained consistent during repeated stimulation for the duration of the experiments.

Silver bipolar electrodes were used to record from the surface of the crista terminalis in an area activated earliest during spontaneous sinoatrial conduction (Sano and Yamagishi, 1965; Bouman et al., 1978). An additional surface electrode was placed on the atrial muscle adjacent to the inferior aspect of the crista terminalis. Transmembrane potentials were recorded using standard microelectrodes filled with 3 m KCl. The recording arrangement is presented in Figure 1A.

In the experiments in which the activation sequence of the sinus node was mapped, the time of activation of the crista terminalis bipolar electrogram site was used as a reference. In some experiments, a stable transmembrane potential recording could be obtained throughout the procedure, and this served as an additional time reference. The mapping was performed by using surface landmarks.
and moving the microelectrode across the sinus node area in a grid of 500-μm increments. In the primary pacemaker area, impalements were made in 200- to 300-μm increments. Between 40 and 50 cell impalements were obtained for each map. The total mapping procedure took from 30 to 60 minutes. Surface electrograms and transmembrane potentials were displayed on an oscilloscope and stored on a Hewlett-Packard 9825A calculator and 9864A digitizer system. Time intervals were measured to the most rapid portion of the depolarization phase of the action potentials or to the peak of the most rapid portion of the intrinsic deflection of the bipolar electrograms. The analyzed data were plotted with a Hewlett-Packard 9872A calculator-plotter. Our system had a time resolution of within 500 μsec.

In our experiments, we will refer to regions of the sinus node as either the primary pacemaker area or the subsidiary pacemaker area. The primary pacemaker area includes those cells which were activated earliest (within 20 msec) during the normal spontaneous rhythm. In these cells there was a smooth transition from diastolic depolarization to the upstroke of the action potential. The subsidiary pacemaker cells in our studies were activated later in the normal sequence of activation and usually exhibited a rapid transition from diastolic depolarization to the upstroke of the action potential.

The potassium activity in the extracellular space of the sinus node was measured using double-barreled potassium-sensitive microelectrodes. The potassium-sensitive electrodes were prepared by filling the tip of one barrel of a double-barreled glass micropipette with a siliconizing agent. A column 200 μm long of the potassium-selective resin (Corning liquid resin no. 477317) injected into the tip was supported by the siliconizing agent. The potassium-selective barrel was then backfilled to the top of the resin column with 100 mM KCl. The second barrel of the microelectrode was used as a reference electrode and was filled with Tyrode's solution. The electrodes had outside tip diameters of 3–4 μm. The potassium-sensitive barrel had a tip resistance of about 1000 MΩ, and the time constant of its response to a rectangular test pulse was 15–20 msec. The reference barrel had a response time constant of 0.2 msec. Silver-silver chloride junctions were used to couple the potassium-sensitive barrel and the reference barrel to high input impedance amplifiers (1.5 × 10¹² Ω). The amplifiers were capacitance compensated and the signals were subtracted by a third difference amplifier (Bloom Associates, Ltd.). The signal from the difference amplifier was the potassium potential (V_K) free of extracellular potential fluctuations which were common to both barrels. The 20-msec response time constant of the potassium-sensitive barrel is 100 times faster than the rise time of the potassium potentials that we measured. The signals from the potassium-selective barrel and the indifferent reference barrel, together with the difference signal, were displayed on an eight-channel oscilloscope and stored on a Hewlett-Packard instrumentation oscilloscope and recorded on a Honeywell 5600C FM tape recorder. The data were also photographed on 35-mm film or recorded on paper at 100 cm/sec with a Mingograph Cardirex 6T recorder. The analog records were digitized and analyzed using a Hewlett-Packard 9825A calculator and 9864A digitizer system.

**Figure 1** A schematic of the isolated rabbit atrial preparation (A) and selected analog records demonstrating the effect of brief vagal stimulation (B). In A, SVC indicates the superior vena cava, AO indicates the aorta, IVC indicates the ostium of the inferior vena cava, and CS indicates the ostium of the coronary sinus. TV is the tricuspid valve. The stippled area is the sinus node. VS indicates the approximate location of the bipolar vagal stimulating electrodes. CT indicates the crista terminalis recording bipolar electrode, and AM is the atrial muscle bipolar electrogram. A control spontaneous interval between CT activations before vagal stimulation is indicated by a; a shortened interval by b. 1, and 1, indicate the time interval from vagal stimulation to the CT activation and the interval between CT activations measured to generate the data point indicated by 1 in Figure 2A; 2, and 2, indicate similar intervals measured to generate the data point indicated by 2 in Figure 2A.
Results

In Figure 1B are analog records obtained during brief submaximal vagal stimulation in an isolated sinus node preparation. SN1 is a transmembrane potential recording from the dominant pacemaker site of the sinus node. SN2 is a recording from a subsidiary pacemaker cell. The time of occurrence of the crista terminalis (CT) activation for the first beat following vagal stimulation was delayed by 582 msec beyond the expected activation time. The next interval was shortened by 41 msec (compare a and b).

The technique originally described by Brown and Eccles (1934) for plotting the time course of the effect of a brief vagal stimulation is also presented in Figure 1B. The coupling intervals (time intervals between successive CT activations) of 15 to 40 beats are plotted vs. the time after vagal stimulation. The method for plotting the first two beats in a sequence following vagal stimulation is shown below the records in Figure 1B. The first interval between CT activations indicated by 1, is plotted vs. the time from vagal stimulation to the activation of the crista terminalis for the beat closing the interval (1). The interval between CT activations for the next beat (2) is plotted vs. the time after vagal stimulation (2).

The complete time course plot for this experiment is shown in Figure 2A. The plot was generated by calculating the intervals between CT activations vs. time for 15 beats after each vagal stimulation. For this figure, there were eight tests with vagal stimulation with the timing of the stimulus set at different and randomly selected coupling intervals relative to the spontaneous sinus beats. Numbers 1 and 2 indicate the data points generated by the first two beats following the vagal stimulation shown in Figure 1B. The bimodal time course of vagal effects is apparent in this plot. This preparation also exhibited a prominent undershoot with coupling intervals shorter than control during the acceleratory phase of the time course of vagal effect. In addition, the reproducibility of the vagal effect for successive vagal stimulations can be seen by the tight grouping of data points in the plot of Figure 2A. Table 1 summarizes the data generated by each experiment. Of the 11 isolated rabbit sinus node-vagal preparations used in these experiments, a bimodal inhibitory curve was observed in six at submaximal stimulus intensities (experiments 1-6). An undershoot with coupling intervals shorter than control spontaneous rhythm during the acceleratory component was observed in four of the six preparations. Five of the preparations had single peak inhibitory curves (experiments 7-11).

In our experiments on vagal effects on sinus rhythm, the studies were carried out with propranolol added to the superfusate at a concentration of 1 mg/liter to eliminate the β-adrenergic responses to stimulation. In four preparations, stimulation in the same area of the superior vena cava was performed with atropine (1 mg/liter) added to the bath instead of propranolol. The atropine blocked the vagal effect and allowed a sympathetic influence on the sinus node. In contrast to the previously described results, in these cases only an acceleratory response was obtained. An example is shown in Figure 2B and the data are summarized in Table 1. The latency between the time of stimulation to the first appearance of acceleration in the spontaneous sinus rhythm was between 800 and 1200 msec. This contrasts with a latency of 170-250 msec for the vagal effect. The maximum acceleration in the presence of atropine occurred between 4.4 and 5 seconds after stimulation, and rate returned to control values between 14.5 and 16 seconds. In contrast, the time course of vagal effect peaked between 0.5 and 0.9 second. The shortest coupling intervals during the acceleratory phase occurred between 0.8 and 1.6 seconds and the secondary slowing peaked between 1.5 and 3.5 seconds. The total time course of vagal effect lasted between 2.4 and 8.0 seconds. The
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Table 1: Time Course of Vagal and Sympathetic Effects

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Interval from stim. to initial slowing (sec)</th>
<th>Interval from stim. to initial maximum (sec)</th>
<th>Interval from stim. to secondary slowing (sec)</th>
<th>Interval from stim. to 90% recovery (sec)</th>
<th>Control basic cycle length (msec)</th>
<th>Maximum slowing (% change from control)</th>
<th>Sinoatrial conduction time (msec)</th>
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<tbody>
<tr>
<td>1</td>
<td>0.21</td>
<td>0.90</td>
<td>1.4</td>
<td>3.0</td>
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<tr>
<td>2</td>
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<td>0.52</td>
<td>0.7</td>
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<tr>
<td>3</td>
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<tr>
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<td>0.75</td>
<td>0.8</td>
<td>3.2</td>
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<td>612</td>
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<tr>
<td>5</td>
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<td>0.52</td>
<td>0.8</td>
<td>3.5</td>
<td>3.5</td>
<td>475</td>
<td>50.3</td>
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<tr>
<td>6</td>
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<td>0.63</td>
<td>1.0</td>
<td>1.6</td>
<td>5.0</td>
<td>469</td>
<td>38.2</td>
</tr>
</tbody>
</table>

Mean ± SD: 0.21 ± 0.03, 0.64 ± 0.16, 0.92 ± 0.26, 2.3 ± 1.0, 5.0 ± 2.0, 511 ± 85.5, 59.4 ± 23.6, 58.5 ± 24.4

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Interval from stim. to initial acceleration (sec)</th>
<th>Interval from stim. to minimum (sec)</th>
<th>Interval from stim. to 90% recovery (sec)</th>
<th>Control basic cycle length (msec)</th>
<th>Maximal acceleration (% change from control)</th>
</tr>
</thead>
<tbody>
<tr>
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<td>450</td>
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<tr>
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<tr>
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<td>16.0</td>
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<td>9.1</td>
</tr>
<tr>
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<td>5.0</td>
<td>15.0</td>
<td>402</td>
<td>7.2</td>
</tr>
</tbody>
</table>

Mean ± SD: 0.96 ± 0.19, 4.6 ± 0.3, 15.4 ± 0.7, 422 ± 75.9, 12.9 ± 5.6

Stim. = stimulation.

time courses for both the vagal effect in A and the sympathetic effect in B of Figure 2 are remarkably similar to comparable data generated previously in the anesthetized dog (Spear and Moore, 1973). In the presence of both propranolol and atropine in the superfusate, stimulation of the superior vena cava produced no change in spontaneous sinus rhythm.

In Figure 3, the effect of changing the intensity of vagal stimulation on the time course of changes in atrial rate is displayed. In Figure 3A, the time course following a vagal stimulation of 0.2 mA is presented. The inhibition time course had a single peak which reached a maximum of 17% change from control and recovered by 90% in 1 second. In the same preparation at a stimulation intensity of 0.4 mA (Fig. 3B), the initial slowing increased to a maximum of 38% change from control, and recovered in approximately 1 second. Also, following the higher intensity vagal stimulation there was an acceleratory component which undershot baseline and produced a secondary slowing which peaked at 1.6 seconds and returned to control in 5 to 6 seconds. The appearance of the acceleratory component and a prominent secondary slowing therefore was dependent on stimulus intensity. The bimodal response persisted even at a supramaximal stimulus intensity. However, obvious shifts in pacemaker location also occurred at this intensity. In Table 1, those preparations exhibiting a single-peaked time course at stimulus intensities producing 50-60% of maximal slowing (numbers 7-11) also had single-peaked responses at supramaximal stimulus intensity.

The effect of vagal stimulation on conduction time in the presence of propranolol was also investigated. Sinoatrial conduction times in the presence of propranolol were 56.1 ± 20.0 msec in our preparation (Table 1). The time course of vagal effect from Figure 2A is replotted in Figure 4A. In Figure 4B, plotted on the common time axis is the conduction time from the primary pacemaker site (SNi) to the CT. Selected analog records are presented in Figure 4C. It can be seen that vagal stimulation produced complex changes in the conduction time of the beats exiting the sinus node. The greatest slowing of conduction within the sinus node occurred within 350 msec after the vagal stimulation (within the time interval defined by a). The total sinoatrial conduction time from the SNi recording site to the CT recording site (B) increased from a control value of 70 msec to 180 msec for the maximally slowed beat at 300 msec following vagal stimulation (A). This was an increase of 110 msec. The increase in CT-CT interval for this beat was 111 msec. Therefore the increased CT-CT intervals for the initially slowed atrial beats between 170 and...
FIGURE 3 The influence of the intensity of vagal stimulation on the time course of changes in atrial rate in the isolated rabbit sinus node-vagal preparation. The coupling intervals between crista terminalis activations were plotted for 22 consecutive beats following two intensities of vagal stimulation delivered as 4-msec pulses at 100 Hz for 120 msec. In A, the intensity of vagal stimulation was 0.2 mA. In B in the same preparation, the intensity of stimulation was 0.4 mA. Maximal stimulation in this preparation produced a maximal slowing of automaticity of 75% change from control.

330 msec following vagal stimulation (a) are explained primarily by slowing of the sinoatrial conduction time. The beats between 330 and 860 msec following vagal stimulation (bracketed in b) include beats with CT-CT intervals that were either maximally increased or maximally decreased. The total sinoatrial conduction time (the conduction interval between SN1 and CT) for the beat at 810 msec with the maximally increased CT-CT interval, increased from a control of 70 msec to 74.4 msec. The sinoatrial conduction time for the beat at 860 msec with the maximally decreased CT-CT interval increased from 70 to 87 msec. The beat at 760 msec exited the sinus node with the shortest sinoatrial conduction time of 56 msec. For beats bracketed in c, the sinoatrial conduction times again were slowed whereas CT-CT coupling intervals were still shorter than or equal to control. Conduction times were not different from control during the secondary slowing of CT-CT interval in this preparation.

In Figure 4C are selected analog records from one vagal stimulation sequence shown by the arrows in A and B. The vertical lines indicate the points of maximum depolarization for the SN1 action potentials, and the numbers indicate coupling intervals for the SN1 and CT responses. Whereas the timing of the first SN1 pacemaker response following vagal stimulation was not significantly delayed, sinoatrial conduction time for this beat was prolonged, producing delayed CT activation.

To investigate more completely the interaction between sinus node automaticity and sinoatrial conduction time following vagal stimulation, we carried...
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Figure 5 presents data from one study. The analog records in A are a transmembrane potential recording from the pacemaker site within the sinus node and a CT electrogram. The influence of vagal stimulation (VS) on automaticity and conduction time out of the sinus node are displayed in A for several beats. The spontaneous cycle length before vagal stimulation was about 470 msec (beat 1). Conduction time between the sinus node pacemaker site and CT activation was 38 msec. Following vagal stimulation, the sinus coupling interval was increased to 635 msec while the coupling interval for the crista terminalis was increased to 647 msec (beat 2). The difference between intervals was due to the fact that the conduction time from the pacemaker site to the CT increased from 38 to 50 msec. The next beat following the vagal stimulation (beat 3) had a coupling interval and conduction time that were shorter than control. It can be seen that the short CT coupling interval (453 msec) was due to the fact that the spontaneous sinus node coupling interval was reduced by 2 msec, as compared to control (468 msec vs. 470 msec), and the fact that beat 2 conducted to the CT 12 msec slower than control, while beat 3 conducted 3 msec faster than control.

Figure 5B presents the sinus node activation sequence map for the three beats indicated in A. The schematic in the upper left quadrant of B indicates the locations of the 38 recording sites used to generate this map. The site of earliest activation was first localized during normal sinus rhythm before the mapping sequence was begun. This assured that during the vagal stimulation test sequences a shift in the site of origin of activity could be related to the control site. In the primary pacemaker area, microelectrode impalements were made at 200- to 300-μm increments so that a shift in the origin of activity could be resolved within 200 to 300 μm. The time and intensity of vagal stimulation was held constant, and each microelectrode recording was made sequentially during stimulations repeated at 1- to 2-minute intervals. In the upper righthand quadrant of B the conduction sequence for beat 1, before vagal stimulation, is shown. It took approximately 25 msec for the impulse to exit the pacemaker area to the surrounding subsidiary pacemaker area. It required an additional 10-15 msec to activate the CT. This sequence of conduction is essentially similar to that previously described by other investigators (Sano and Yamagishi, 1965; Bouman et al., 1978). It can be seen that the area exhibiting the slowest conduction is within the pacemaker area of the sinus node. In the lower lefthand quadrant of B, the conduction sequence for beat 2 is shown. For this beat, the site of earliest activity was shifted 200-300 μm superiorly, but the origin of the impulse was still within the primary pacemaker area. This shift cannot account for the acceleratory component of the vagal time course. However, there was a prominent slowing of conduction out of the pacemaker site as compared to the prestimulation state (beat 1). It can be seen that the additional slowing of conduction from the pacemaker site to the CT associated with the vagal stimulation occurred within the sinus node pacemaker area itself. It required 35 msec to exit the
pacemaker area and an additional 10-15 msec to activate the CT. In the lower right quadrant, the conduction sequence for beat 3 is plotted. In this case, the conduction time was slightly shorter than control values. This is not apparent in the conduction sequence map for this beat since the map resolves 5 msec intervals. However, in A, the analog records indicate beat 3 took 35 msec to activate the CT.

For the microelectrode impalement shown in A, the interval between sinus node beats 2 and 3 was reduced by 2 msec compared to control. For each of the 38 recording sites used to generate the maps in B there also was a reduction in the interval between beats 2 and 3 associated with vagal stimulation. Because of the sinoatrial conduction slowing for beat 2, the reduced interval between beats 2 and 3 became more pronounced as impalements were made more peripherally in the sinus node. Sites within the sinus node adjacent to the CT exhibited reduced intervals between beats 2 and 3 of 17 msec less than control intervals.

The mapping studies verify that for low intensity stimulation the acceleratory component of the time course of vagal effect does not necessarily rely on reentry or a shift in the pacemaker site for its mechanism. Although the effect of vagal stimulation on the sinoatrial conduction time can contribute to the acceleratory component of the time course of vagal effect, there is a decreased coupling interval for the sinus node pacemaker site itself.

The action potential changes responsible for the rate changes in the sinus node pacemaker are apparent in Figures 1B and 5A. The initial slowing of sinus rate in the bimodal time course of vagal effect was due to an increase in the maximum diastolic membrane potential and a decrease in the slope of diastolic depolarization. Changes in "threshold potential" cannot be definitely determined because of the smooth transition between diastolic depolarization and action potential upstroke. The secondary slowing in the sinus rate was due to a decrease in the slope of diastolic depolarization with little change in the maximum diastolic potential. The acceleratory component was due to a transient decrease in the maximum diastolic potential following the hyperpolarization associated with the initial slowing. During this time there was a variable effect on the slope of diastolic depolarization in our experiments with some cases showing a slight decrease and others showing a slight increase.

In all of our preparations that exhibited an acceleratory component, it was consistently found to be due to a transient decrease in the maximum diastolic potential of the cells within the pacemaker region of the sinus node. In Figure 6 are data localizing this phenomenon to a specific region within the sinus node. Analog records presented in Figure 6A demonstrate the results of a vagal stimulation (VS) on transmembrane potentials from the

![Figure 6](http://circres.ahajournals.org/)

**Figure 6** The effect of vagal stimulation on the maximum diastolic potential of cells of the rabbit sinus node. SN<sub>1</sub> is a record obtained from the primary pacemaker region of the sinus node. SN<sub>2</sub> is a record obtained from a subsidiary pacemaker fiber, and CT is an electrogram recorded from the crista terminalis in an area excited earliest in the normal sequence of atrial activation. In the analog records, horizontal lines indicate the control maximum diastolic potential. Vagal stimulation was performed at the time indicated by VS. The changes in maximum diastolic potential were determined for the coupling interval indicated by the asterisk for the two cells shown in A as well as 44 additional recording sites. The timing and intensity of vagal stimulation was held constant for each of these sequential recordings. In B, a schematic of the sinus node is presented. The area bounded by the dashed lines indicates the region from which sinus node action potentials were recorded. SN<sub>1</sub> and SN<sub>2</sub> indicate the recording sites for the corresponding analog records presented in A. The plus signs in the schematic indicate those sites in which the maximum diastolic potential was depolarized at the time indicated by the asterisk in A. The minus signs indicate those sites in which the maximum diastolic potential was hyperpolarized at the time indicated by the asterisk in A. The zeros indicate sites which showed no deviation from control maximum diastolic potential at this time. These data are from the same experiment as presented in Figure 5.
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The pacemaker region of this preparation (SN1) and from a more peripheral subsidiary pacemaker site (SN2). In both the SN1 and SN2 recordings, hyperpolarization immediately followed vagal stimulation, and the coupling interval was increased. However, the second beat following the vagal stimulation occurred at a shortened coupling interval. This corresponds to the acceleratory component of the time course of vagal effect. It can be seen that the SN1 cell in the pacemaker area was depolarized during this interval, whereas the SN2 cell remained hyperpolarized following the vagal stimulation (indicated by asterisks). Localization of the depolarization to the primary pacemaker area of the sinus node was characteristic of all of our preparations. In the schematic in B are data from 46 consecutive transmembrane potential recordings within the sinoatrial node. In this preparation, the time of vagal stimulation was held constant for each test while the microelectrode was moved to sample the various areas. The plus signs indicate that the recording site exhibited a depolarization following vagal stimulation at the time corresponding to the asterisk in A. The minus sign indicates the cell impaled exhibited a hyperpolarization. A zero indicates no deviation of the maximum diastolic potential from control value at that time. A localization of the depolarization phenomenon to the area of the primary pacemaker in this preparation is apparent in the schematic (compare Figs. 5B and 6B).

In Figure 7 is shown the time course for changes in the maximum diastolic potential following vagal stimulation for a subsidiary pacemaker fiber (A) and a sinus node primary pacemaker fiber (B). The data were generated by plotting the change in maximum diastolic potential for several beats following vagal stimulation. The timing of vagal stimulation relative to the spontaneous cycle was changed and the procedure repeated until a complete time course was generated. The data in A obtained from a subsidiary pacemaker fiber indicate only a hyperpolarization following vagal stimulation. The peak hyperpolarization occurred at 250 msec and subsided from the maximum $-14.4$-mV change to control in about 1.7 seconds. In B, for the sinus node primary pacemaker cell, there was an initial $-7.9$-mV hyperpolarization. However, within 500 msec after vagal stimulation, there was a shift to a depolarization of $8.2$ mV. The maximum diastolic potential returned to control within 1.9 seconds. While the total duration of the effect was comparable in both sinus node primary pacemaker fibers and more peripheral subsidiary pacemaker fibers, the pattern of the changes in maximum diastolic potential following vagal stimulation were distinctly different for these two types of sinus node cells. The maximum hyperpolarization tended to be greater in subsidiary pacemaker cells, and depolarization was observed only near the primary pacemaker area (Table 2).

Potassium-sensitive microelectrodes were inserted into the extracellular space of the sinus node to investigate the influence of the accumulation of potassium in the extracellular space following vagal stimulation on the time course of changes in spontaneous atrial rate. In Figure 8 are analog records from one experiment. In A, vagal stimulation of relatively strong intensity was delivered to the preparation. This was sufficient to prolong the initial coupling interval by 192%. The recording from the sinus node pacemaker cell indicates that, following this intense vagal stimulation, there was a shift in the site of the primary pacemaker. In this experiment the time course of the vagal effect was single peaked. The high intensity stimulation was necessary in this preparation to produce a consistent and large accumulation of potassium. In the potassium...
TABLE 2  A Comparison of the Changes in Maximum Diastolic Depolarization following Vagal Stimulation

<table>
<thead>
<tr>
<th>No. of preparations</th>
<th>No. of impalements</th>
<th>Maximum hyperpolarization (mV)</th>
<th>Maximum depolarization (mV)</th>
<th>Time to recovery (sec)</th>
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<tbody>
<tr>
<td>Subsidiary pacemaker</td>
<td>8</td>
<td>-13.0 ± 6.1*</td>
<td>4.7 ± 2.0</td>
<td>2.0 ± 0.3</td>
</tr>
<tr>
<td>Primary pacemaker</td>
<td>6</td>
<td>-7.1 ± 6.4</td>
<td>4.7 ± 2.0</td>
<td>1.8 ± 0.3</td>
</tr>
</tbody>
</table>

* Mean ± SD.

electrode record ($V_K$), the potential across the resin at the tip of the electrode increased following vagal stimulation to a peak of 1.0 mV in 2.0 seconds. This represents an increase of potassium in the extracellular space of 0.17 mM. The accumulated potassium was cleared from the extracellular space in 14-16 seconds. In B, the addition of atropine (0.2 mg/liter) eliminated both the rate changes and potassium changes following vagal stimulation. In three experiments in which extracellular potassium transients were recorded following vagal stimulation, the resulting time courses were very similar. Following a total of 70 vagal stimulations in the three preparations, the time to peak accumulation ranged between 1.4 and 2.5 seconds, and the halftime for recovery from the peak accumulation ranged between 2.8 and 4.6 seconds.

The consistency of the potassium transient following vagal stimulation is shown in Figure 9C. The means and standard deviations of the potassium activity in millimoles per liter were calculated at 500-msec intervals following vagal stimulation for 10 consecutive tests performed at 1- to 2-minute intervals. The latency between vagal stimulation and the beginning of the potassium accumulation was 0.70 ± 0.06 second. The peak accumulation occurred at 2 seconds and returned to control at 13 to 14 seconds following vagal stimulation. The clearing of the potassium from the extracellular space followed a monotonic exponential time course with a time constant of 3.32 seconds and a r square value of 0.99. In addition, plotted on the common time axis in Figure 9 are a typical single-peaked time course (A) and a typical bimodal time course (B) obtained from two additional preparations. The basic cycle lengths and maximum percent change in slowing following vagal stimulation were comparable in both cases. By comparing Figure 9, A and B, it can be seen that in those preparations exhibiting a single-peaked inhibitory time course (A), the duration of the inhibition corresponds to the duration of the initial slowing of the bimodal time course (B). This observation, together with the data of Figure 3, suggests that the bimodal time course is not simply the result of the difference between an acceleratory influence and a faster rising, longer duration inhibitory influence as previously suggested (Iano et al., 1973). If this were the case, when one observed a single-peaked time course of vagal effect, presumably a situation in which the acceleratory component is absent, the duration of the time course should extend into the

![Figure 8](https://example.com/figure8.png)

**Figure 8**  Analog records obtained using a potassium-sensitive microelectrode positioned in the extracellular space of the sinus node. CT is a crista terminalis bipolar electrogram. SN is the transmembrane potential recording from the dominant pacemaker area of the sinus node. $V_K$ is a recording using a potassium-sensitive microelectrode placed in the extracellular space adjacent to the SN recording site. VS indicates the timing of vagal stimulation consisting of 4-msec pulses at 100 Hz delivered at 2-mA intensity for 300 msec. The records in A were obtained in the presence of propranolol (1 mg/liter) added to the superfusate. In B the records were obtained 15 minutes after the addition of atropine (0.2 mg/liter) to the bath.
The time course of the accumulation of potassium in the extracellular space (C) following vagal stimulation plotted on a common time axis with a single-peaked (A) and double-peaked (B) inhibitory time course. The ordinates in A and B are the coupling intervals of crista terminalis activations for 20 and 25 sequential beats following vagal stimulation. The ordinate in C is the calculated potassium activity in mM/liter. The data points in C are the means of 10 measurements made during 10 runs of vagal stimulation. The standard deviation for each data point is also shown. A, B, and C are from different preparations. The equation which describes the exponential recovery of the potassium activity following the peak accumulation is $y = 0.133 e^{-t/0.5}$. 

Discussion

There is reason to believe that the magnitude of the changes in extracellular potassium activity as measured by the potassium electrode may be smaller than the actual intercellular changes (Lux and Neher, 1973). This arises because the potassium electrode tip may distort the extracellular space. The sinus node cells are tightly packed and the extracellular space may be only 100-150 angstroms wide (Kawamura, 1961). A double-barreled potassium electrode with a tip diameter of 3-4 μm must therefore be resting in an artificially distorted extracellular space. The ratio of the active membrane surface area to the volume that it surrounds could therefore be as much as 500 times smaller than that for an undistorted extracellular cleft. This would result in a dilution of the potassium as it flows into the extracellular space where the electrode tip rests. The actual magnitude of the extracellular potassium transient may therefore be underestimated.

It is known that the ion exchange resin used for sensing the potassium ion is also sensitive to acetylcholine in the range of $10^{-7}$ to $10^{-4}$ molar (Lux, 1974). One study suggests that the plasma concentration of acetylcholine necessary to produce a 30% change in sinus rate is $10^{-6}$ molar (Levy and Zieske, 1969). To verify that the acetylcholine released by vagal stimulation was not responsible for the changes in our potassium records, we added atropine to the superfusate at a concentration of 0.2 mg/liter and restimulated the vagus with the same stimulus intensity. The results of this experiment are shown in Figure 8B. Following the application of atropine over a period of 10 minutes, both the rate changes associated with vagal stimulation as well as the magnitude of the changes in extracellular potassium accumulation were gradually eliminated with the same time course. Since atropine acts at the postsynaptic membrane, acetylcholine presumably was still released in response to vagal stimulation. If the acetylcholine were causing the changes in the potassium electrode record, we would have expected them to persist in the presence of the atropine. The elimination of the change in potential of the potassium electrode by atropine in B verifies that the potential changes that we observed in A were due to an increase in potassium ion at the potassium electrode tip.

Our experiments were performed on an isolated rabbit atrial preparation in which autonomic stimulation was applied at the superior vena cava. This eliminates the extensive dissection necessary to iso-
late free-running vagal or sympathetic fibers. The preparation has a unique advantage in that it remains stable and exhibits consistent responses to autonomic stimulation for prolonged periods. This allows one to study the effects of autonomic stimulation on both sinoatrial conduction and automaticity using sequential microelectrode impalements rather than multiple simultaneous recordings. Our data provide a new mechanism for the double-peaked inhibitory response of the sinus node to vagal stimulation first described by Brown and Eccles (1934). We also present indirect evidence defining the kinetics of acetylcholine's action following vagal stimulation as well as the underlying ionic mechanisms responsible for the double-peaked inhibitory time course of changes in atrial rate following vagal stimulation. The role of the accumulation of potassium in the extracellular space in these rate changes can be inferred.

The data demonstrate that submaximal vagal stimulation does not shift the origin of activity out of the primary pacemaker area but still produces a bimodal time course with an acceleratory component. The shortened atrial coupling intervals following vagal stimulation are due to decreased coupling intervals of the primary pacemaker site within the sinus node. In addition, vagally induced changes in sinoatrial conduction time contribute to the pattern of changes in atrial rhythm following vagal stimulation. The greatest percentage of the slowing in sinoatrial conduction occurred within the sinus node primary pacemaker area. The initially prolonged atrial coupling intervals within 170-330 msec after vagal stimulation are prolonged primarily due to sinoatrial conduction slowing or change in the exit pathway with very little change in the coupling interval of the primary pacemaker (Fig. 4).

The kinetics of the acetylcholine transient following vagal stimulation can be derived from three observations. First, in Figure 3A following a low intensity vagal stimulation, the inhibition was over in approximately 1 second. Also, in those preparations which exhibited only a single-peaked time course (Fig. 9A and Table 2), the single-peaked response recovered by 90% at 1.84 ± 0.74 seconds after vagal stimulation. Second, the membrane hyperpolarization (Fig. 7A) which is a result of the acetylcholine-mediated increase in membrane potassium conductance (Burgen and Terroux, 1953) subsided in 2.0 ± 0.3 seconds. Third, Figure 9C demonstrates that the accumulation of potassium in the extracellular space peaked within 2 seconds after vagal stimulation. Thus, if we assume that the initial slowing of sinus rate in the single-peaked time course is a direct effect of acetylcholine, and if we further assume that the hyperpolarization and accumulation of extracellular potassium are due to the direct actions of acetylcholine on increasing the potassium conductance, we can conclude that the acetylcholine released by the vagal stimulation was inactivated at the receptor site within approximately 2 seconds after stimulation.

In Figure 3B, the intensity of vagal stimulation was increased in the same preparation previously showing a single-peaked response. The timing of the initial slowing was approximately the same, but now an acceleratory component and a secondary slowing appeared. Whereas the acceleratory component occurred within the time of action of acetylcholine, the secondary slowing occurred when acetylcholine was presumably inactivated. Therefore the secondary slowing probably was not the result of a direct action of acetylcholine.

Our data suggest a possible ionic mechanism for the acceleratory component. In Figure 6 and Table 2 it can be observed that areas in the sinus node respond differently to vagal stimulation. In the subsidiary pacemaker area, vagal stimulation produced only a hyperpolarization. The primary pacemaker area within the sinus node showed a smaller hyperpolarization, as well as a prominent phase of depolarization of the maximum diastolic potential. In Figure 7 and Table 2 it can be seen that the time courses for both the hyperpolarization in the subsidiary pacemaker area and the depolarization in the primary pacemaker area of the sinus node show a return to control within approximately 2 seconds following stimulation. The studies of Toda and West (1967) and Burke and Calaresu (1972) suggest that the action of acetylcholine on the primary pacemaker cells of the sinus node involves an increase in the membrane's sodium conductance as well as an increase in the potassium conductance. Whereas the increased potassium conductance would be expected to cause a hyperpolarization, the increased sodium conductance would exert a depolarizing influence. Our data localizing the depolarization effect to the true pacemaker area of the sinus node (Fig. 6) and the fact that the time course of recovery from depolarization (Fig. 7) corresponds to our indirectly determined kinetics for the inactivation of acetylcholine support the idea that the depolarization we observed within the pacemaker area of the sinus node was due to an acetylcholine-mediated increase in the sodium conductance. Also, we cannot rule out the possibility that the potassium which was accumulating in the extracellular space at this time also contributed to the depolarization. The time course of the depolarization (Fig. 7B) corresponds remarkably well to the time course of the "acceleratory component" of the double-peaked inhibitory vagal response described by Iano et al. (1973).

The acceleratory phase in the bimodal time course is due to depolarization of the maximum diastolic potential of the primary pacemaker cells resulting in an early attainment of threshold potential. The presence or absence of an acceleratory phase in the time course in a given condition or preparation should therefore depend on the relative...
magnitudes of the depolarizing effect and the hyperpolarizing effect. In Figure 3B, when the intensity of vagal stimulation was increased, the acceleratory component appeared, and presumably this resulted from the pacemaker cells being sufficiently depolarized to cause the shortened coupling intervals.

If acetylcholine is essentially inactivated in 2 seconds following vagal stimulation, one must look for an explanation other than a direct action of acetylcholine for the secondary slowing of the sinus node. The phase of secondary slowing of sinus rate peaks from 1.0 to 3.5 seconds following vagal stimulation and returns to control within 3.5 to 8 seconds. The data of Figure 9, B and C, indicate a possible mechanism for the secondary slowing of sinus rate. The accumulation of potassium in the extracellular space is coincident with the secondary slowing. Since the secondary slowing is due to a decreased rate of diastolic depolarization without a change in maximum diastolic potential, it is possible that this depression of diastolic depolarization may be a direct result of the increased extracellular potassium. However, in experiments on isolated rabbit atria (Toda and West, 1967) and guinea pig atria (Antoni et al., 1963) in which potassium concentration was increased in the superfusate, both maximum diastolic membrane potential and rate of diastolic depolarization of sinus node cells were decreased by potassium concentrations of up to 10 mM/liter, and little change in sinus rate occurred. Above 10 mM/liter, there was a decrease in sinus rate. If the accumulated potassium in the extracellular space following vagal stimulation is the cause of the decreased rate of diastolic depolarization in our experiments, then the sensitivity of the sinus node to this intrinsic potassium transient must be different from that when potassium concentration is increased in the superfusate. A second possibility is that the accumulated potassium in the extracellular space stimulates an electrogenic ion pump which acts to slow the rate of diastolic depolarization (Noma and Irisawa, 1974). Experiments on isolated rabbit atrial muscle in which an accumulation of potassium in the extracellular space was induced by rapid pacing provided evidence for an active potassium ion pump. There was a transient undershoot in the potassium ion concentration in the extracellular space after it had returned to control following the termination of rapid pacing in these experiments (Kunze, 1977).

The present experiments as well as previous data from our laboratory (Kronhaus et al., 1978) provide evidence for a complex mechanism for the changes in spontaneous atrial rate following brief vagal stimulation. The bimodal time course is a result of changes in the intrinsic rate of the pacemaker site itself, as well as changes in sinoatrial conduction time.

The various components of the bimodal time course may be explained by our data as follows. The initial slowing of atrial rate which occurs within 170 to 300 msec after vagal stimulation (i.e., just beyond the latency for an effect) is due to slowing of conduction or change in exit pathway out of the sinus node. The greatest part of the initial slowing is due to a direct action of acetylcholine on the sinus node membrane producing a decrease in the rate of diastolic depolarization, as well as a hyperpolarization of the maximum diastolic potential. The acceleratory component when it is present is due to depolarization of the maximum diastolic potential possibly because of an increased membrane conductance to sodium and occurs exclusively within the primary pacemaker area of the sinus node. Both the initial slowing of automaticity and the acceleratory component therefore are direct actions of acetylcholine. The secondary slowing of sinus rate appears to be coincident with the accumulation of extracellular potassium following vagal stimulation. The relative contribution of these various components would be expected to vary from preparation to preparation (Table 1) as well as be dependent on the parameters of vagal stimulation (Fig. 3). Variations in the basic pattern of the bimodal time course may therefore be explained in this way.

Acknowledgments

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Contractures and Increase in Internal Longitudinal Resistance of Cow Ventricular Muscle Induced by Hypoxia

JACEK WOJTCAK

SUMMARY This study was performed to determine whether hypoxia in glucose-free solutions can increase the electrical resistance of intercellular junctions in ventricular muscle. Internal longitudinal resistance (Ri), mechanical tension, and transmembrane action potentials were measured simultaneously in cow ventricular trabeculae. The mean control value of Ri was 265 ± 38 Ωcm (mean ± SE) at 34°C. After 1 hour of hypoxia in glucose-free Tyrode's solution, it had increased by 300 ± 41% (n = 11, P < 0.001). The rise in Ri was closely related to the increase in resting tension (contracture). These effects were more pronounced during a second exposure to hypoxia and were potentiated by application of epinephrine, by increasing extracellular calcium concentration, and by increasing frequency of stimulation. Addition of glucose (50 mM) provided some protection against hypoxia. It is inferred that the increase in Ri is entirely due to the increase in the resistance of intercellular junctions (electrical uncoupling). Intracellular calcium may be responsible for both the contracture and the uncoupling.

Circ Res 44: 88-95, 1979

THE influence of hypoxia and ischemia on the electrical activity of cardiac cells has been the subject of many investigations (Trautwein et al., 1954; Trautwein and Dudel, 1956; MacLeod and Daniel, 1965; MacLeod and Prasad, 1969; McDonald and MacLeod, 1973; Friedmann et al., 1973). However, these studies were limited to the measurement of cellular resting and action potentials, and little is known about the changes in passive electrical properties. It would be interesting to establish whether the resistance of cell-to-cell electrical junctions could be modified by hypoxia, because such changes can be of relevance to the development of arrhythmias during myocardial infarction.

The intercellular junctions in heart muscle are formed by nexus, which are responsible for the
The effect of brief vagal stimulation on the isolated rabbit sinus node.
J F Spear, K D Kronhaus, E N Moore and R P Kline

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