Phasic Effects of Repetitive Vagal Stimulation on Atrial Contraction

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SUMMARY. The vagus nerves of anesthetized dogs were stimulated once each cardiac cycle with a brief burst of pulses, and the timing of the stimulus bursts was changed by a fixed increment on successive cardiac cycles. The effects of such vagal stimulation on atrial contraction depended on the number of pulses per stimulus burst, on the interval between pulses within the burst, and on the timing of the stimulus bursts within the cardiac cycle. The vagal stimulus bursts had the least negative inotropic effect when they were given less than 100 msec before the next atrial depolarization. This dependence of the inotropic response on the timing of the vagal activity within the cardiac cycle indicates that the following conditions must prevail with respect to the vagal innervation of the atrium: (1) the acetylcholine released from the vagal nerve endings is hydrolyzed at a critically rapid rate in the atrial tissues, (2) the neurally released acetylcholine must exert its major influence on atrial contraction during some preferential fraction of the cardiac cycle (presumably, during depolarization), and (3) after a vagal stimulus of a given strength, the concentration of acetylcholine in the region of the myocardial cells will attain its maximum value during this critical phase of the cardiac cycle when the vagal stimuli are given at the optimal time in the cardiac cycle. (Circ Res 52: 657–663, 1983)

SPONTANEOUS efferent vagal activity to the heart is usually characterized by brief bursts of impulses that are synchronized with each cardiac cycle (Jewett, 1964; Katona et al., 1970; Kunze, 1972). The timing of such bursts of vagal activity relative to the cardiac cycle is an important determinant of the heart rate (Levy et al., 1969, 1970, 1972, 1978; Dong and Reitz, 1969, 1970, 1972, 1978; Martin, 1975). A change in timing of only 5 to 10 msec can result in large changes in the magnitude of these cardiac responses. The present experiments were conducted to determine whether the timing of repetitive bursts of vagal activity had similar phasic effects on the inotropic response of the atria.

Methods

Ten dogs (14–25 kg) of both sexes were anesthetized with pentobarbital sodium (30 mg/kg, iv). A tracheal cannula was inserted through a midline cervical incision. The cer-

Electrodes from a second Grass stimulator were con-

The atrial electrogram (A wave) was recorded from an
electrode in the tip of the left auricle. This signal was
c connected to an analog computer (EAI-580), where it was
to derive the cardiac cycle duration (A-A interval) with
a resolution of 1 msec. All experimental and derived vari-
bables were recorded on an oscillograph (Brush Mark 200)
and on analog magnetic tape (Honeywell 7600).

The vagus nerves were stimulated with one burst of
square wave pulses each cardiac cycle (Grass stimulator and
isolation unit, model 54KR). For all experiments, the pulse
amplitude of the vagal stimuli was 10 V, and the pulse
width was 1.0 msec; these stimulus parameters are supra-
maximal (Levy et al., 1978). The interval (A-St) from the
beginning of the A-wave to the onset of the stimulus burst
was controlled by the analog computer. The A-St interval
was changed by a constant value of 10 to 20 msec in
successive cardiac cycles; i.e., the A-St interval was alter-
nately increased and decreased as ramp functions of time
(Fig. 1). Each ramp was swept through the entire cardiac
cycle before the direction was reversed.

Electrodes from a second Grass stimulator were con-

ected to the left auricular appendage to pace the heart, so
that the atrial contractile responses could be measured at
constant contraction frequencies. The left auricle was se-
lected for pacing to avert the possibility that the neurotrans-
mitters that would be released by the pacing stimuli could
have no appreciable influence on the measured inotropic
responses of the right auricle. The heart was paced at two
cardiac cycle durations, one just below the spontaneous
cycle duration, and the other at a cycle duration that was 100–200 msec less than that value.

We determined the effects of the following factors on atrial contraction: (1) the timing (A-St) of the vagal stimuli; (2) the number (n) of pulses in the vagal stimulus bursts (2 or 6 for most animals); (3) the interval (I) between consecutive pulses within the vagal stimulus bursts (5 or 25 msec); and (4) the atrial pacing interval (A-A).

Results

Representative Experiment

Figure 1 shows the effects of varying the phase (A-St interval) of the repetitive bursts of vagal stimulation on atrial contraction in a representative experiment. Varying the A-St intervals as a sequence of ascending and descending ramp-like functions of time evoked concomitant changes in the amplitude of the atrial contractions. When there were six pulses in each burst of vagal stimuli (Fig. 1, D–F), the atrial contraction amplitudes were considerably less than the amplitudes that prevailed when there were only two pulses per burst (A–C). Also, when there were only two pulses per burst, the alterations in amplitude evoked by changes in the phase (A-St) of the vagal stimuli were considerably greater than when there were six pulses in each burst.

Changing the atrial pacing interval from 400 to 500 msec had little effect on the amplitude of contraction (compare panels A and D with panels B and E, respectively), but it did affect the waveshape of the response. At the longer A-A interval (500 msec, panels B and E), there was a second, lower peak of contractile amplitude that occurred when the A-St interval was close to zero. However, this second, lower peak was absent (panel A) or very small (panel D) when the pacing interval was only 400 msec. This effect of shortening the pacing interval was consistent in all animals.

When the interpulse interval was 25 msec, the contractile amplitude was less than when the interpulse interval was only 5 msec (compare panels B and E with panels C and F, respectively). This difference was more pronounced when there were six pulses/burst (panels E and F) than when there were only two pulses/burst (panels B and C).

The precise effect of stimulating the vagi at a given phase (A-St interval) of the cardiac cycle depended on whether the A-St interval was being varied as an ascending or as a descending ramp; i.e., the response displayed significant hysteresis. In Figure 2, the changes in atrial contraction shown in panel B of the preceding figure are plotted as function of the A-St interval. Note that, for any given A-St interval, the amplitude of the atrial contraction during the ascending ramp was greater than that during the descending ramp. A very similar hysteresis loop was obtained for the data of Fig. 1-A; i.e., when the A-A interval was 400 msec.

Composite Results

Figure 3 illustrates how the following seven response variables were quantified for analysis of the composite results: (1) MAX is the maximum amplitude of the atrial contraction tracing, (2) MIN is the minimum amplitude of the contraction tracing, (3) DIFF is the difference between MAX and MIN, (4) A-St:max is the A-St interval (time from the beginning of atrial depolarization to the beginning of the vagal stimulus burst) for the stimulus burst that evokes the MAX contraction, (5) St-A:max is the difference between the prevailing A-A interval and A-St:max (i.e., the interval between the stimulus and the next atrial depolarization, for the stimulus burst that evokes the

![Figure 1](https://example.com/f1.png)

**Figure 1.** The effects of one burst of vagal stimulation per cardiac cycle on atrial contraction in an anesthetized dog. The time interval (A-St interval) from the beginning of atrial depolarization to the beginning of the burst of vagal stimuli was varied by a constant amount each cardiac cycle, such that the changes in A-St interval were varied as sequences of ascending and descending ramps. In this experiment, the number (N) of pulses in each stimulus burst was either 2 (panels A–C) or 6 (panels D–F); the interval (I) between individual pulses in the burst was either 5 or 25 msec. The atria were paced at cycle lengths (A-A intervals) of either 400 or 500 msec.

![Figure 2](https://example.com/f2.png)

**Figure 2.** A plot of atrial contraction amplitude as a function of the A-St interval for the data shown in Figure 1B. The atrial contraction amplitudes obtained during the ascending ramps of A-St intervals are indicated by the rightward arrows; those obtained during the descending ramps, by the leftward arrows.
Figure 3. A schematic representation of the different response variables that were measured or computed. These variables are described in detail in the text.

MAX contraction), (6) A-St: min is the A-St interval for the stimulus burst that evokes the MIN contraction, and (7) St-A: min is the difference between the prevailing A-A interval and A-St: min.

Figure 4 shows how the first three of the above response indices were affected by the cardiac cycle duration (A-A), the number (n) of pulses per burst and the interpulse interval (I). At least two replicates of each response index were obtained for each combination of the experimental factors. All possible permutations of the various levels of the three factors (two levels of A-A, three levels of N, and three levels of I) were applied in a random sequence. A mixed-model analysis of variance (Sokal and Rohlf, 1969) was performed, where these three factors were considered to be the fixed factors, and the individual animals and the replicate observations were considered to be random factors.

The maximum (MAX) and minimum (MIN) contraction amplitudes and the peak-to-peak fluctuations (DIFF) all decreased significantly (P < 0.01) as the number (n) of pulses/burst of vagal stimuli was increased and as the interval (I) between pulses was increased (Fig. 4). The maximum amplitude and the peak-to-peak fluctuation also decreased significantly (P < 0.05) as the cardiac cycle duration (A-A) was diminished. The minimum amplitude also tended to decrease as the cardiac cycle duration was reduced, but the change was not statistically significant. There were no statistically significant interactions between any of the variables, as determined by the analysis of variance.

Frequency histograms were constructed for the St-A intervals that were associated with the minimum (St-A: min) and the maximum (St-A: max) amplitudes of the atrial contractions (Figs. 5 and 6). The data from all animals at all 18 combinations of experimental factors were pooled for each histogram. The minimum contraction occurred with the greatest frequency when the St-A interval equalled 330 msec (Fig. 5). The maximum amplitude of contraction occurred with the greatest frequency when the St-A interval was between 0 and 100 msec (Fig. 6).

Discussion

Phase-Dependent Effects

Our experiments show that when the vagi are stimulated with one brief burst of pulses during each cardiac cycle, the negative inotropic effect on the atria is significantly influenced by the timing of those
stimulus bursts within the cardiac cycle. In order for such a phase dependency to be manifest, the following three conditions must prevail: (1) the concentration of acetylcholine (ACh) in the neuroeffector gaps within the cardiac tissues must change substantially over the time course of each cardiac cycle, (2) the responsiveness of the effector cells to a given concentration of ACh must vary appreciably during the cardiac cycle, and (3) the response of the effector cells at some critical period within the cardiac cycle must depend on the prevailing ACh concentration in the neuroeffector gaps.

It is obvious that if one brief burst of vagal stimulation each cardiac cycle resulted in a concentration of ACh in the neuroeffector gaps that did not vary appreciably during the cardiac cycle, the cardiac response would not depend on the timing of the vagal stimulus bursts. It is already well established that the chronotropic responses of the sinoatrial and atrioventricular (SA and AV) nodes and the dromotropic responses of the AV conduction system show a pronounced phase dependency to vagal stimulation (Levy et al., 1969, 1970, 1972, 1978; Dong and Reitz, 1970; Martin, 1975; Wallick et al., 1979). This is understandable in that these responses begin to appear at about 200 msec, and reach a peak at about 400–500 msec, after the stimulus. However, the inotropic response to a brief vagal stimulus burst does not begin until about a second after the stimulus, and the peak response occurs several seconds after the stimulus burst and lasts for several seconds (Martin, 1980). Despite the relative slowness of this response, the present experiments reveal that such a phase dependency applies to the vagal control of atrial contraction, just as it does to various other aspects of cardiac function.

These findings indicate, therefore, that the ACh concentration in the neuroeffector gaps must vary periodically (i.e., with the same period as the cardiac rhythm) under the conditions of these experiments. Such periodicity signifies that (1) the release of ACh from the postganglionic vagal nerve endings must occur as relatively brief discharges when the vagal activity occurs in short bursts, and (2) the neurally released ACh must be dissipated at a rate sufficiently rapid that its concentration declines appreciably within the time course of a single cardiac cycle.

The principal mechanisms for removal of the neurally released ACh are diffusion and hydrolysis (Lindmar et al., 1982). This latter process is catalyzed by acetylcholinesterase, an enzyme that is distributed throughout the cardiac tissues, but that is especially abundant in the nodal structures (James and Spence, 1966; James, 1967). The results of the present study indicate that these dissipative processes are sufficiently effective to achieve a significant change in ACh concentration in the atrial myocardium within the time limits of one cardiac cycle. Other factors being equal, the shorter the cardiac cycle, the less would be the magnitude of the change in ACh concentration over the course of a cardiac cycle. It may be inferred that such differences in ACh concentration, at least partially, account for the significantly greater peak-to-peak fluctuation (DIFF) in contractile amplitude that we observed at the longer than at the shorter cardiac cycle length (Fig. 4). In the perfused chicken heart, the half-time for elimination of ACh from the interstitial spaces was about 2.5 seconds (Lindmar et al., 1982).

As stated above, a second prerequisite for phase dependency is that the responsiveness of the effector cells must vary during the cardiac cycle. Consider first that brief bursts of stimuli (Sta) are applied to the vagus nerves near the end of each cardiac cycle (i.e., shortly before the atrial depolarization that terminates the cycle). After an appropriate latent period, the ACh concentration in the neuroeffector gaps in the atrium would rise quickly to a maximum value, and then would decline throughout the remainder of the cardiac cycle. Consider that the timing of the stimulus and the duration of the latent period were such that the peak ACh concentration occurred during the atrial action potential.

Now consider that identical stimuli (Stb) are given to the vagus nerves each cardiac cycle, but at some time after, rather than shortly before, the onset of atrial depolarization. The changes in ACh concentration in the neuroeffector gaps evoked by repetitive stimuli Stb would be identical to those evoked by Sta, except for a phase shift equal to the time lag between Sta and Stb. It is apparent that if the response of an atrial myocardial cell to a given instantaneous concentration of ACh were the same at all times in the cardiac cycle, then the inotropic response of that atrial cell to stimuli Sta would be identical to the response to stimuli Stb.

The response of an atrial myocardial cell to a given instantaneous ACh concentration does vary considerably with the time in the cardiac cycle, however. The action of ACh on atrial contraction is mediated
almost exclusively during the depolarization phase of the transmembrane action potential. ACh modulates atrial contraction by altering the influx of calcium into the cell during the plateau phase of the action potential, principally by decreasing the duration of the action potential (Ten Eick et al., 1976). When the concentration of ACh is sufficiently great, this transmitter may also diminish the slow inward current per unit time of plateau (Ten Eick et al., 1976), which effect would contribute to the reduction in Ca influx.

Thus, one series of stimulus bursts (StA), each applied at a critical time in the cardiac cycle, would exert a much greater negative inotropic effect than would another series of stimuli (StB). The two series were identical in all respects except in their timing. Stimuli StA are located at that critical interval prior to depolarization such that the maximum ACh concentration would prevail in the neuroeffector gaps during (or just before) the plateau phase of the action potential. The most effective time probably would be shortly before the plateau, because there is an appreciable latent period that extends from the interaction of ACh with the muscarinic receptor till the actual change in the membrane characteristics of the cardiac cell (Hill-Smith and Purves, 1978; Osterrieder et al., 1981).

Vagal stimulus bursts with an St-A interval of 330 msec (St-A:min) were most likely to induce the minimum atrial contraction amplitude (Fig. 5). Hence, this was the St-A interval that exerted the maximum negative inotropic effect. This interval comprises the following components: (1) conduction time in the efferent vagal fibers; (2) synaptic delay between the preganglionic and postganglionic vagal fibers; (3) the time for the ACh concentration in the neuroeffector gaps to rise from the minimum to the maximum value, and (4) the latent period from the time that ACh interacts with the muscarinic receptors till the appearance of the first detectable change in the characteristics of the myocardial cell membrane. The time required for components (1) and (2) is only about 20 msec (Brown and Eccles, 1934). A recent study by Osterrieder et al. (1981) indicates that component (4) occupies about 30-80 msec in rabbit S-A nodal cells. Hence, the time for the ACh concentration to rise from its minimum to its maximum value (component 3) is therefore estimated to be about 230-280 msec (i.e., the difference between the observed St-A:min and the sum of the other factors). In isolated rabbit S-A node preparations, when ACh was released ionophoretically, the time to reach peak hyperpolarization varied from 170 to 1000 msec, depending on the quantity of ACh that was released (Osterrieder et al., 1981).

We previously showed that a single burst of vagal activity did not produce any inotropic response until one to three beats after the stimulus, depending on the heart rate. The peak effect was reached on the third to fifth beat after the stimulus. A near maximal effect lasted for several subsequent beats. This relatively slow time course indicates that there is a considerable lag between a given change in ACh concentration and the atrial inotropic response to that change in concentration. Nevertheless, a single vagal stimulus pulse does have an absolute maximal effect on only one of the subsequent beats 3, 4, or 5 (Martin, 1980).

Thus, the phase of the stimulus burst given in each cardiac cycle is an important determinant of the contractile response, but only after a latent period of several seconds. The A-St interval in the present work was changed slowly, so that the timing of the ACh peak concentration and delayed maximal inotropic response to that peak were in a relatively steady state from beat to beat. Thus, the above interpretation of the present data is consistent with our previous work on the inotropic effects of single stimulus pulses.

Effects of the Number of Pulses/Burst and of the Interpulse Interval

When the number of pulses in each burst of vagal stimuli was increased, the amplitudes of the atrial contractions diminished (Fig. 4). Undoubtedly more ACh was released from the vagal terminals during a given stimulus burst as the number of pulses included in that burst was increased. This would have raised the concentration of ACh in the neuroeffector gaps, and therefore it would have induced the observed augmentation of the negative inotropic effect of the vagal stimulation (Fig. 4).

The greater negative inotropic effect of increasing the interpulse interval (Fig. 4) was probably also ascribable to an increase in the ACh concentration in the neuroeffector gaps. Our previous studies (Levy et al., 1978) have shown that the negative chronotropic responses to vagal stimulation also increased as the interpulse interval was increased, over the range of interpulse intervals from 3 to 30 msec. It is likely that the quantity of ACh released from the nerve endings with each stimulus pulse diminishes when the time between pulses is inadequate. Perhaps less ACh-containing granules are able to move up to the secretory membrane of the varicosity and be released when the time between neuronal action potentials is too short. Also, a prejunctional negative-feedback mechanism appears to be involved in the regulation of ACh release (Kilbinger, 1977). As the concentration of ACh rises in the immediate vicinity of the postganglionic vagal nerve endings, there is a greater inhibition of the release of ACh from those same endings (Kilbinger, 1977). In general, the closer together in time the neuronal action potentials occur, the greater will be the instantaneous concentration of ACh in the neuroeffector gap, even though the quantity of ACh released per burst of pulses may be reduced. Hence, as the time between stimulus pulses is increased, the negative feedback signal tends to diminish, and more ACh is released in response to each pulse.

Hysteresis Effects

The data from the representative experiment shown in Figures 1 and 2 indicate that when the A-St intervals of the vagal stimulus bursts were progressively...
increasing, the inotropic responses were appreciably different from those that were elicited during progressively decreasing A-St intervals; i.e., the response displayed considerable hysteresis. Note that the atrial contraction at a given A-St interval during an ascending ramp of A-St intervals was greater than the atrial contraction at that same A-St interval during a descending ramp (Fig. 2). Note also that the atrial contractions that occurred at the end of an ascending ramp of A-St intervals were considerably greater than those that were observed at the end of the previous or the following descending ramp (Figs. 1 and 2). If hysteresis did not prevail, the atrial contractions at these two points in the cycle should have been virtually equal, because the timing of the vagal stimuli within the cardiac cycle is almost identical at the two ends of the ramps; i.e., the stimuli were delivered within a few milliseconds of the onset of atrial depolarization. Hence, the peak of the curve of ACh concentrations should have been at almost the identical point in the cardiac cycle, regardless of whether the A-St interval corresponded to the bottom or to the top of the ramp.

We verified this assertion in a few experiments by triggering the change in the direction of the ramp at another point in the cardiac cycle (at the beginning of ventricular depolarization). Under these new experimental conditions, the bursts of stimuli moved without interruption past the time of the onset of atrial depolarization. We confirmed that there was no sudden change in the amplitude of atrial contractions when the phase of the stimulus burst was shifted from just before to just after the beginning of atrial depolarization, or vice versa.

One reason for the hysteresis is that the true vagal stimulation frequency was different during the ascending and descending ramps. The time from the beginning of one vagal stimulus burst till the next stimulus burst equals (A-A) + (A-St), where (A-A) is the pacing cycle length and (A-St) is the change in phase from cycle to cycle. During the ascending ramps, (A-St) is positive, whereas during the descending ramps, (A-St) is negative. Hence, the time between stimulus bursts during ascending ramps was greater than that which prevailed during descending ramps. Therefore, the vagi were stimulated with more bursts each minute during descending ramps than during ascending ramps. For this reason, the negative inotropic effect of the descending ramps would be expected to exceed that of the ascending ramps. The hysteresis loop shown in Figure 2 conforms to this prediction.

We may speculate that the hysteresis is also ascribable to the substantial time that is required for the activator pool of calcium in the sarcoplasmic reticulum of the atrial myocardial cells to reach a steady state value after a relatively abrupt change in Ca++ influx across the myocardial cell membrane (Wood et al., 1969; Morad and Goldman, 1973; Winegrad, 1979). Consider, for example, that the timing of the bursts of vagal stimuli has been shifted from the point in the cycle where it has its greatest inhibitory effect to the point in the cycle where it exerts a much weaker negative inotropic effect. The Ca++ influx into the cell will suddenly increase, but substantial time is required before the activator pool of Ca++ will have risen to its new steady state level. To the extent that the size of the activator pool of Ca++ determines myocardial contractility, therefore, the response (contraction amplitude) will tend to lag the stimulus (change in A-St interval). At the rates of change of A-St intervals that were implemented in our experiments (10 to 20 msec/cycle), sufficient time was probably not permitted for the activator pool of Ca++ to reach the steady state value during any given cardiac cycle that would be appropriate for the A-St interval that prevailed for that cycle. Such a lag therefore would be likely to lead to hysteresis.

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