Phase-Related Sensitivity of the Sinoatrial Node to Vagal Stimuli in the Isolated Rat Atrium

Sherry L. Stuesse, Matthew N. Levy, and Harrison Zieske

SUMMARY In isolated rat atria, endogenous neurotransmitters were released by electrical pulses that were below threshold for activation of the myocardial cells. A brief train of pulses was delivered at a specified time after each atrial activation to determine the relationship between the time of stimulus delivery during an atrial cycle (P-St interval) and the subsequent cardiac cycle length (P-P interval). These stimuli caused a change in P-P interval from a mean control level of 291 msec to a mean maximum P-P interval of 592 msec. This change was attributed to the release of neurotransmitters from sympathetic and parasympathetic fibers, but the effects from the parasympathetic nerve terminals predominated. Increasing the number of pulses per burst increased the atrial slowing. The extent of slowing was dependent on the time of stimulus delivery in an atrial cycle. The region of minimal effectiveness (mean P-P interval of 412 msec during stimulation) was obtained with P-St intervals that were only slightly greater (about 10 msec) than those that were maximally effective. Stimuli delivered at times in the cardiac cycle that fell between the P-St intervals that elicited maximum and minimum changes in heart rate occasionally caused profound irregularities in heart rate. These changes in cycle length do not appear to be due to pacemaker shifts, but are probably the result of the interaction between acetylcholine and the sinoatrial nodal cell membrane. The responsiveness of this membrane to acetylcholine changes with time during the cardiac cycle.

SEVERAL laboratories have demonstrated that heart rate varies with the time of vagal stimulus delivery during a cardiac cycle. Over a certain range of stimulation frequencies, an increase in frequency caused an increase in heart rate instead of the expected decrease (the "paradoxical" effect of vagal stimulation). Previous experiments have used whole animals, and the vagi were stimulated at a considerable distance from the heart. The underlying basis for this phase dependency of neural stimulation is still unresolved. Endogenous neural transmitters can be released from the heart by direct electrical stimulation at voltages that do not excite the myocardial cells. We have applied this technique of neurotransmitter release to investigate the phase-dependent sensitivity of the sinoatrial node to acetylcholine in the isolated right atrium of the rat. With this preparation the effect of selective stimulation of the nerve fibers in the sinoatrial (SA) nodal region itself may be examined, and the factor of variable neural conduction times encountered during stimulation of the vagal trunks in the neck is averted. Very brief bursts of subthreshold stimuli were applied to the SA nodal region, resulting in the release of small amounts of endogenous neurotransmitter during a cardiac cycle. The increase in cycle length was related to the time of stimulus delivery. This phase dependency is characterized in this paper, and it is compared with the phase dependency elicited by cervical vagal stimulation in intact dogs.

Methods

Atrial Preparation

White male rats (300–500 g) were injected, ip, with sodium pentobarbital (3 mg/100 g). The heart was excised...
and washed in physiological saline solution. The right atrium, with part of the superior and inferior venae cavae, was cut from the heart and pinned to a paraffin platform in a chamber containing 75 ml of saline solution: 145 mM NaCl, 5.4 mM KCl, 2.2 mM CaCl2, 11.9 mM NaHCO3, 11.0 mM glucose. The pH was adjusted to 7.4 with dilute HCl. The solution was gassed continuously with 95% O2 and 5% CO2 and was maintained at 34–35°C.

**Stimulation and Recording**

The preparation was viewed through a dissecting microscope. With the aid of a micromanipulator, a bipolar stimulating electrode made from silver wires (0.13 mm in diameter, Teflon-coated except at the tips) was positioned on the epicardial surface of the atrium slightly anterior to the midpoint of the superior vena cava-atrial margin. An electrical signal, which will be referred to hereafter as the "stimulus," was delivered to the isolated atrial preparation from two Grass S9 stimulators connected in series. The first stimulator served as a gate to control the total duration of the stimulus (7–70 msec). The second stimulator delivered square-wave pulses (30 μsec in duration, 100–200 Hz) to the atrium. Duration and voltage of the pulses were kept below threshold for direct excitation of myocardial pacemaker cells, but the pulses were suprathreshold for some of the nerves in the atrium. Such stimuli will be referred to as "subthreshold" stimuli in this paper, using the terminology of Vincenzi and West. 8

The myocardial threshold value was determined empirically for each electrode position on the atrium. A stimulus consisted of either a single brief pulse or a train of brief pulses, depending on the frequency of stimulation and the total stimulus duration. For most of the experiments reported in this paper, one such stimulus was delivered at a preset time after each atrial activation. In a few experiments (those reported in Figure 5), one stimulus was delivered at random every 20 beats.

The atrial electrogram (P wave) was recorded with a second bipolar silver electrode which was embedded in the atrial appendage. The atrial signal then was filtered (½ amplitude frequencies: 3 Hz low, 0.3 kHz high), amplified with a Grass AC preamplifier, and recorded on an eight-channel oscillograph (Brush, Mark 200). The filtered atrial signal also triggered an analog computer, which timed the delivery of the stimulus (St) at any preset time relative to the P wave, with an accuracy of 1 msec. The P-St interval is the time from the beginning of the P wave to the beginning of the stimulus; it was varied experimentally by turning a potentiometer. The analog computer was used to determine the time interval from one P wave to the next (P-P interval). The P wave, the stimulus event marker, the P-P interval, and the P-St interval were recorded on the oscillograph. In a few experiments, a second bipolar electrode was used to record an electrogram from another site on the atrium in order to detect any pacemaker shifts.

**Location of the Region of Maximum Responsiveness**

To locate the region of the greatest density of vagal fibers to the SA node, the stimulating electrode was positioned at the intercaval margin, and a short train of stimuli (for example, 40 msec) was delivered to the preparation during every atrial beat. The electrode was moved, and the preparation was explored for the region that gave the greatest prolongation of the P-P interval during stimulation. This region was generally at the intercaval margin of the superior vena cava slightly anterior to the midpoint between the two caval ostia. When the stimulating electrode was moved just a few millimeters, heart rate no longer changed with stimulation. Changes in chronotropic responses were not observed when regions on the superior or inferior vena cava other than the caval margins were stimulated. In a blood-perfused, isolated preparation, Kubota and Hashimoto9 determined the chronotropic responses to subthreshold stimulation of various portions of the dog atrium. We found that the location of the area that evokes the greatest slowing of the heart rate in the rat is very similar to that which they observed in the dog.

After the most responsive location had been identified, the voltage and pulse duration of the stimuli were adjusted to give as large a change in atrial rate as possible while still keeping the intensity subthreshold for the myocardium. In about 20% of the atria studied, it was not possible to get a chronotropic change of at least 25% using subthreshold stimuli, and these atria were not studied further.

**Drugs**

Drugs used and their final concentrations were: atropine sulfate (0.4 μg/ml), propranolol HCl (1.3 μg/ml), and the ganglionic blocking agents hexamethonium chloride (20–100 μg/ml), pentolinium tartrate (Ansolysen, 1.3–2.0 μg/ml), and trimethaphan camyslate (Arfonad, 67–670 μg/ml). Drugs were added in small volumes (less than 0.5 ml) directly to the bathing solution containing the atrium, and the effects were tested at intervals from 2 to 30 minutes after administration.

**Results**

**Response from the Atrium, One Stimulus per Beat**

A single stimulus or brief train of stimuli was applied once each beat to the region of the SA node very early in the cardiac cycle (P-St = 0). The P-P interval was abruptly prolonged (first arrow, Fig. 1). With repetitive stimulation at the same P-St interval, a steady state P-P interval was reached within a few seconds. In about a third of the atria, varying the P-St interval caused only a negligible change in the P-P interval. In the majority of atria, however, as the stimulus was delivered progressively later in the atrial cycle, the atrial rate continued to slow until a P-St interval was reached at which the P-P interval suddenly became unstable (second arrow, Fig. 1). The instability consisted of alternate short and long P-P intervals, and it disappeared as the P-St interval was increased slightly. At this point (third arrow, Fig. 1) the P-P interval was less than that observed at shorter P-St intervals, but greater than the prestimulation cycle length. From this minimum P-P interval, as the stimulus was delivered later and later in the cycle, there was
another gradual small increase in P-P interval. This response pattern could be reversed by reversing the direction of the ramp of P-St intervals. During stimulation, the P-P interval was always greater than the spontaneous P-P interval in the absence of stimulation. When stimulation was stopped (fourth arrow, Fig. 1), the P-P interval decayed to the control level in 3-10 beats.

Pacemaker Response Curves

Curves were constructed in which the time of impulse delivery (P-St) was related to the subsequent change in P-P interval. Such "pacemaker response curves" graphically display the phase-dependent sensitivity of the SA nodal region to vagal stimuli.1,3-5 Pacemaker response curves from a single atrium are shown in Figure 2 for various stimulation conditions. A repetitive subthreshold stimulus of two pulses per burst given during the atrial cycle was sufficient to cause a marked prolongation of the P-P interval (closed circles, Fig. 2A; horizontal dashed line represents prestimulation P-P interval). The amount of prolongation depended on the time of stimulus delivery, which is expressed as absolute time following the preceding P wave (P-St interval). The P-P interval was the greatest at a P-St interval of 0.18 sec and least when the stimulus was delivered just 0.01 sec later. When the number of pulses per burst was increased to four, the P-P interval was even more prolonged at most P-St intervals, but the shape of the curve was basically the same (open circles, Fig. 2A). The drop from the apex to the trough of the pacemaker response curve occurred over a small fraction of the cardiac cycle in virtually every preparation studied. In this atrium, no beat irregularities were apparent when either two or four stimuli were delivered per burst.

For a given voltage and burst duration, maximal P-P prolongation was achieved when 6-9 pulses were delivered per burst (open and filled circles, Fig. 2B). A further increase in the number of pulses per burst did not cause any noticeable increase in P-P interval (triangles, Fig. 2B). Thus, 6-9 pulses per burst seemed to be sufficient to activate maximally all the intrinsic nerve fibers in the nodal area which could be excited at the voltage used.
Irregularities in the atrial cycle (similar to those in Fig. 1, second arrow) were apparent in the region of the pacemaker response curve with a negative slope (discontinuities in curves in Fig. 2B). The P-St interval over which these irregularities appeared was relatively fixed for a given preparation, and this range did not increase as the number of pulses in the stimulus burst increased. However, increasing the number of stimuli per burst did increase the likelihood of these oscillations. Thus none were seen when two to four pulses per burst were delivered (Fig. 2A), but they were evident in the same preparation when six or more pulses per burst were given (second arrow in Fig. 1 and bracketed regions in Fig. 2B).

Mean Pacemaker Response Curve

To give an overview of the effect of parasympathetic stimulation on cardiac cycle length in the isolated atrium under our experimental condition, a composite pacemaker response curve was constructed (Fig. 3). Mean P-P values derived from 13 atria which gave near maximal responses, using the subthreshold stimulation technique, are shown at various stimulation times relative to the atrial P wave (P-St intervals). The bars represent either plus or minus the SE of the mean (only one direction is shown for clarity). The mean spontaneous cycle duration in the absence of neural stimulation was 0.29 ± 0.02 sec (horizontal dotted line, Fig. 3). The stimulus burst had its greatest effect (B, Fig. 3) on prolongation of the P-P interval at a mean P-St interval of 0.30 ± 0.02 sec, which was virtually identical to the control P-P interval. Vagal stimulation at this P-St interval caused approximately a 2-fold increase in cycle length (P-P = 0.59 ± 0.12 sec). When the stimulus burst occurred about 0.01 sec later relative to atrial activation, the stimulus was minimally effective (P-P interval = 0.42 ± 0.07 sec). The effectiveness then increased approximately linearly as the stimulus was delivered later in the cardiac cycle. When the stimulus occurred at the very end of a cycle (just before the beginning of a P wave), the mean P-P interval from the 13 atria was 0.44 ± 0.02 sec. This mean P-P value was plotted at a P-St interval of 0.44 sec, and also at a P-St interval of approximately zero, since the P-P interval was virtually identical when the stimulus occurred slightly later, just after the beginning of the P wave (P-St = 0). Thus, the ability of acetylcholine to slow heart rate varied in a reproducible manner from cycle to cycle. During stimulation, the P-P intervals at the points shown in Figure 3 were always greater than the control interval (P < 0.001, using a paired t-test). In addition, the maximum and minimum P-P intervals during stimulation were significantly different from each other (P < 0.001).

Response from the Atrium, 1 Stimulus/20 Beats

In three experiments, one stimulus was delivered during a cardiac cycle every 20 beats. The time of stimulus delivery during a cardiac cycle was randomly selected. The relationship between the time of random stimulus delivery (measured from the time of stimulus delivery to the subsequent P wave: St-P) and its effect on that cycle in which it was delivered (ΔP-P) is illustrated in Figure 4. When the stimulus was delivered early in the cycle, it caused a prolongation of the cycle in which it was delivered. If the stimulus was delivered late in the cycle, it had no effect on the cycle in which it was delivered, but prolonged subsequent cycles. It can be seen that at the end of the cycle there is a "latent" period of about 80 msec during which the stimulus had no effect on the cycle.
in which it was delivered. In three atrial preparations, the latent period ranged from 80 to 90 msec in duration.

**Pharmacological Characterization**

Most of the experiments reported in this paper were carried out on atria which had been exposed to neither sympathetic nor parasympathetic blocking agents. Two separate effects on the frequency of atrial activation can be attributed to the subthreshold stimulation. The first is the marked increase in cardiac cycle length described in the preceding sections. The second effect is a decrease in cycle length which ordinarily was not apparent until stimulation had been stopped (Fig. 5A) and which often was not seen (Figs. 1 and 6). The prolongation of the P-P interval could be blocked by atropine (Fig. 5B), indicating that it was caused by stimulation of the intrinsic parasympathetic nerves. In atria that displayed a poststimulation decrease in cycle length, blocking the parasympathetic component allowed this increased rate of beating to be observed during stimulation (Fig. 5B). The onset and decay of this diminished cycle length were much slower than the parasympathetically induced increase in cycle length, an observation consistent with its being mediated through sympathetic nerve fibers. This poststimulation decrease in cycle length could be blocked by propranolol (Fig. 5), further supporting its sympathetic origin. The amount of shortening of the P-P interval did not vary with time of stimulus delivery.

Ganglionic blocking agents were used to determine whether the prolongation of the P-P interval during stimulation was due to pre- and/or postganglionic excitation of the intrinsic parasympathetic nerve fibers. The response from the atria was not changed by any of the following ganglionic blocking agents: hexamethonium chloride, trimethaphan camsylate, or pentolinium tartrate. Thus, after ganglionic blockade, the parasympathetic response was ascribable to stimulation of only postganglionic fibers.

**Search for Pacemaker Shifts**

A characteristic feature of the response to subthreshold stimulation in these preparations was the abrupt change in cardiac cycle length with P-St interval at a critical level of the P-St interval (e.g., second arrow, Fig. 1). One explanation for this abrupt change in the response is that there may be a sudden change in the pacemaker region, perhaps to the atrioventricular (AV) node. To investigate this possibility, 90% of the atrium, including the atrial appendage, the AV node, and the intercaval region near the inferior vena cava, was cut away, leaving only the superior vena cava and a small portion of the atrium, including the SA node. The preparation still showed typical responsiveness to changes in the P-St interval.

A second test of the possibility of a pacemaker shift was to record the atrial electrogram from two separate sites on a whole right atrium (Fig. 6). The timing and shape of the two deflections from these sites were then compared before and during delivery of the stimulus burst at various times in the cardiac cycle. If there was an appreciable shift in the pacemaker site during stimulation, a change in the relationship of the two P waves would have been apparent. No detectable change in time relationship or shape of these two P waves was seen (Fig. 6), even though there were marked shifts in the P-P interval as the P-St interval was varied.

**Discussion**

The present experiments demonstrate that a brief "subthreshold" burst of pulses delivered to an isolated rat atrium has an effect on the cycle length that varies with the time of delivery of that stimulus. This phase dependence seems to be analogous to that seen with vagal or carotid sinus nerve stimulation in intact animals. In both systems, an increase in the number of pulses within each burst causes a greater slowing of heart rate. Increasing the number of pulses per burst also increases the tendency for irregularities in the rate of beating in both systems when the burst is delivered at a critical phase of the cardiac cycle. An abrupt shift in the pacemaker response curve (e.g., from B to A in Fig. 3) can be demonstrated in the isolated rat atrium with just 2 pulses per burst. Sharp changes also were observed in intact dogs during cervical vagal stimulation, but 6–10 pulses per burst usually were necessary. In both preparations, increasing the number of pulses per burst increases the amplitude of the change from the maximum to the minimum P-P interval. A stimulus delivered to the cervical vagus in the intact animal results in impulses that travel with a variety of conduction times in individual efferent fibers. Thus one would expect the action potentials evoked by a stimulus delivered to the cervical vagus to be more dispersed in time when it arrived at the SA node than would those elicited by a comparable stimulus delivered to the vagus at a site much nearer the SA node. This probable dispersion of the action potentials might explain why, with a stimulus of 1–2 pulses per burst, the change in P-P interval was more gradual with cervical vagal stimulation in the intact animal but was more prominent with direct neural stimulation near the SA node in the isolated preparation. It is likely that any change in pacemaker cell responsiveness resulting from a change in the phase of the cardiac cycle would be resolved more pre-
cisely the less the distance of the site of vagal stimulation from the SA node.

The mean interval between the time of the abrupt shift in the pacemaker response curve and the beginning of the next atrial P wave may be deduced from Figure 3. The minimum cycle prolongation (A) was 0.42 sec, and it occurred when the P-St interval was 0.30 sec. Thus, the mean interval from the stimulus to the next P wave (the St-P interval) was 0.12 sec. This is denoted as the time from a to c in Figure 7A. The maximum cycle prolongation (B in Fig. 3) was 0.59 sec, and it occurred when P-St was 0.29 sec. Hence, the St-P interval was 0.30 sec; this is denoted as the time from a to c in Figure 7B. Ordinarily, the action potential in the SA nodal cells precedes atrial depolarization. To affect the length of that cardiac cycle in which a vagal stimulus is delivered, the time of stimulus delivery must precede the initiation of the SA nodal action potential that gives rise to the atrial P wave that terminates that cycle. Therefore, at the maximum P-P interval (B in Fig. 3), the beginning of the SA nodal action potential occurred 300 msec or less before the P wave, and at the minimum P-P interval (A in Fig. 3) the time of SA nodal action potential initiation occurred 120 msec or less before the P wave. (The upstroke of the SA nodal action potential occurred at some time between lines a and c in both panels of Figure 7.)

Additional information on the temporal relationship between the SA nodal action potentials and the atrial P waves is provided by the experiments in which the latent period to vagal stimulation was determined. The latent period comprises the time for the excitation of vagal nerve endings, the release of the neurotransmitter, its postsynaptic action, SA nodal action potential generation, intranodal and perinodal conduction, and conduction in the atrium to the site of the P wave recording electrode. This period in our experiments (Fig. 4) was about 80–90 msec. Thus, if the vagal endings in the SA node were excited at least 80 to 90 msec before the beginning of the P wave, that cardiac cycle that included the stimulus was prolonged. Conversely, if the nerve endings were excited less than 80 msec before the P wave, the concurrent cardiac cycle was unaffected, but the subsequent cycle was lengthened. This sets 80–90 msec as the time between the beginnings of the SA nodal action potential and the atrial P wave in our preparation (time between b and c in Fig. 7) when the vagal endings are stimulated only once every 20 beats. This time is probably somewhat longer when the vagal terminals are excited once each heart beat.

Previous investigators have measured more directly the time between the upstroke of the action potential in the SA node and the onset of the P wave, i.e., the

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

**Figure 6** Oscillograph record of P waves recorded from two sites on an atrium: relationship of beat interval and time of stimulus delivery. Site 1 (P₁): slightly above the inferior vena cava ostium. Site 2 (P₂): in upper quadrant of atrium about halfway between SA node and distal edge of atrial appendage. Arrows indicate changes in time base. Fast speed is 100× that of slower time base. For further discussion, see text.
perinodal conduction time (the time between b and c in Fig. 7). The actual value depends on various factors, including the size and conduction properties of the preparation and the location of the atrial recording electrodes. Cramer et al. recorded a time lag of 70 msec between the beginnings of the SA nodal action potential and the atrial P wave in isolated rabbit atria. Other investigators reported shorter conduction times, about 20-40 msec.

In the absence of sudden perturbations, such as a change in neurotransmitter concentration, pacemaker cells in the SA node depolarize at a relatively steady rate during phase 4. When the vagus is stimulated, the concentration of acetylcholine (ACh) at the SA nodal cell membranes is suddenly increased. Our data show that the ability of the ACh to prolong the cardiac cycle varies with time. At one brief portion of the cycle (B-A, Fig. 3), there is a sudden shift in the membrane responsiveness that may be related to a critical level of membrane potential in the SA nodal cells (represented by the horizontal dotted lines in Fig. 7). If a sudden increment in ACh concentration occurs before this critical level of depolarization is reached (point a in Fig. 7B), that cycle in which the vagal stimulus is delivered will be prolonged. This lengthening of the cycle will be achieved by a diminution of the slope of the pacemaker potential during phase 4, and perhaps by a relative hyperpolarization as well.

If the sudden increment in ACh concentration affects the SA nodal cell membranes after the postulated critical level of depolarization has already been reached (point a in Fig. 7A), it will be too late to affect that cycle in which the stimulus is delivered. The stimulus will then affect the subsequent cycle. However, by the beginning of the subsequent phase 4 depolarization, some of the ACh that had been released earlier by the stimulus will have been hydrolyzed. Thus the nodal membrane response during this next cardiac cycle will be diminished. The sooner the stimulus is delivered after the critical level of depolarization is reached, the more time there will be for hydrolysis of ACh, and therefore the less will be the lengthening of the subsequent cardiac cycle. During the occurrence of the SA nodal action potential per se, ACh probably has no tendency to prolong the cardiac cycle, but it may actually have the opposite effect. The recent study by Lipsius and Vassalle demonstrates that ACh significantly decreases the duration of the SA nodal action potential. This influence, by itself, would tend to shorten the cardiac cycle.

Thus, ACh released just before or during the SA nodal action potential would be relatively ineffective in lengthening the cardiac cycle for two reasons; (1) the maximal ACh concentration during the SA nodal action potential would shorten the action potential and, hence, tend to curtail the cardiac cycle, and (2) some of the ACh would be hydrolyzed by the time that phase 4 depolarization begins, and therefore it would be less effective in altering the rate of diastolic depolarization. Conversely, ACh released during the early and middle portions of phase 4 would be relatively more effective in prolonging
the cardiac cycle for the opposite reasons: (1) the ACh concentration would be relatively great during phase 4, thereby having a maximal influence during that period, and (2) some ACh will have been hydrolyzed by the time of the upstroke of the next SA nodal action potential, and hence the tendency to shorten that action potential will be abridged. As phase 4 proceeds, the negative chronotropic efficacy of ACh increases progressively until, at some critical point near the onset of the next pacemaker action potential, the efficacy diminishes abruptly and substantially.

References

Phase-related sensitivity of the sinoatrial node to vagal stimuli in the isolated rat atrium.
S L Stuesse, M N Levy and H Zieske

Circ Res. 1978;43:217-224
doi: 10.1161/01.RES.43.2.217

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1978 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/43/2/217.citation

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/