

Dynamic Vagal Control of Pacemaker Activity in the Mammalian Sinoatrial Node

Jose Jalife, Victor A.J. Slenter, Joseph J. Salata, and Donald C. Michaels

From the Department of Pharmacology, S.U.N.Y. Upstate Medical Center, Syracuse, and The Masonic Medical Research Laboratory, Utica, New York

SUMMARY. Dynamic heart rate control by parasympathetic nervous input involves feedback mechanisms and reflex bursting of efferent cardiac vagal fibers. Periodic vagal bursting induces phasic changes in sinoatrial cycle length and can entrain the pacemaker to beat at periods that may be identical to those of the vagal burst. We investigated the electrophysiological basis of these phenomena in isolated sinus node preparations (rabbit, cat, and sheep). In the presence of propranolol (3.9×10^{-6} M), relatively brief (50–150 msec) trains of stimuli, applied onto the endocardial surface of the preparation, activated postganglionic vagal terminals and induced a brief hyperpolarization of sinoatrial pacemaker cells. This vagally mediated hyperpolarization could alter the pacemaker rhythm by an amount that depended on its duration and its position in the cycle, as well as on the duration of the free-running pacemaker period. When the free-running period was sufficiently long and the hyperpolarization was induced sufficiently early in the spontaneous cycle, a “paradoxical” acceleration of the pacemaker rhythm ensued. Phasic changes were plotted on phase-response curves, constructed by scanning systematically the sinoatrial pacemaker period with single or repetitive vagal trains. These phase-response curves enabled us to predict the entrainment characteristics and the levels of synchronization of the pacemaker to the vagal periodicity. The overall data explain the cellular mechanisms involved in the phasic effects of brief vagal discharges on sinoatrial periodicity, and provide conclusive evidence for the prediction that repetitive vagal input is capable of forcing the cardiac pacemaker to beat at rates that can be faster or slower than the intrinsic pacemaker rate. These data should improve our knowledge of the dynamic control of heart rate by neural reflexes and aid in our understanding of rhythm disturbances generated by the interaction of the cardiac pacemaker with vagal activity. (*Circ Res* 52: 642–656, 1983)

THE control of heart rate by the autonomic nervous system is a dynamic process mediated in part by reflexly induced periodic discharges of efferent cardiac vagal fibers. Each time the systolic pressure wave arrives at the baroreceptor regions in the aorta and the carotid sinuses, it generates a discrete burst of impulses in the efferent fibers (Green, 1959; Iriuchijima and Kumada, 1963). Experiments from several laboratories have demonstrated that periodic vagal stimulation can induce phasic changes in heart rate and can force the cardiac pacemaker to beat at periods that may be equal, or harmonically related, to the period of the vagal input (Suga and Oshima, 1968, 1969; Reid, 1969; Levy et al., 1969). The mechanism of the synchronization phenomenon is explained by the observation that a brief vagal discharge can alter the pacemaker period by a quantity that depends on the magnitude of the stimulus and on the time of its arrival during the cardiac cycle (Levy et al., 1970; Eckberg, 1976; Jalife and Moe, 1979a).

Recently we studied the cellular basis of these phasic phenomena in an isolated vagus nerve-sinus node preparation (Jalife and Moe, 1979a). In that study, brief trains of stimuli applied to the cervical portion of the vagus were used to perturb the sinoatrial pacemaker at different phases of its spontaneous cycle. Vagally induced transient hyperpolarizations resulted in phase shifts of the pacemaker rhythm that

depended on the timing, amplitude, and duration of the hyperpolarization. In addition, sinus node-sucrose gap experiments in which the effects of shaped hyperpolarizing pulses were used to scan the pacemaker period suggested that the magnitude of the phase shift is a function of the relationship between the duration of the hyperpolarization and the duration of the pacemaker period (Jalife and Moe, 1979a). Those experiments provided important clues as to the cellular mechanisms involved in the phasic vago-nodal interactions originally described by Donders (1868) and later plotted by Brown and Eccles (1934).

In the present study, we have employed the isolated sinus node preparation, and we have applied perturbation techniques of oscillator theory (Pinsker, 1977) in an attempt to provide direct evidence for the cellular mechanisms of phasic vago-nodal interactions. These results should improve our knowledge concerning the dynamic control of heart rate by neural reflexes and aid in our understanding of rhythm disturbances generated by the interactions of the cardiac pacemaker with periodic, reflexly mediated, vagal input.

Methods

Definitions

In this paper, the sinoatrial pacemaker is treated as a system of homogeneous, self-sustained oscillators coupled

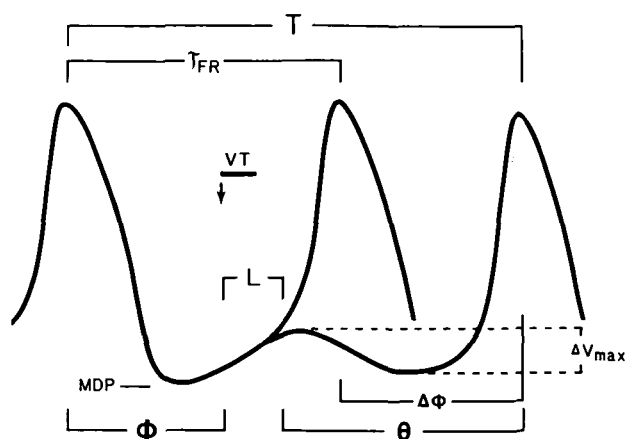


FIGURE 1. Definition of terms; τ_{FR} , free-running period; T , new period resulting from vagal input; VT, vagal train; L , latency; MDP, maximum diastolic potential; ϕ , phase of VT; ΔV_{max} , maximum membrane potential change induced by VT; θ , cophaseness (duration of hyperpolarizing action); $\Delta\phi$, phase shift induced by VT.

to each other by low resistance pathways, and subject to identical levels of vagal innervation. Obviously, this is an oversimplification (c.f. Bleeker et al., 1980). Yet, it is necessary if one is to define the basic behavioral properties of the cardiac pacemaker in response to periodic vagal input. The period (τ ; 1/frequency) of the pacemaker or pacemaker cycle length (PCL) is the time required to complete one full cycle of activity and is measured at the peak of the sinus nodal action potential (Fig. 1). The intrinsic or free-running period (τ_{FR}) of the pacemaker is the period in the absence of external influences (e.g., vagal input). The time within the cycle at which an input is applied will be referred to as the phase (ϕ), and will be expressed in terms of absolute value (msec) or normalized to the free-running period (i.e., $\phi/\tau_{FR} \times 100$).

A brief vagal train (VT) applied at a specific phase in the pacemaker period (Fig. 1) induces a hyperpolarization. This effect begins after a latency (L) associated with activation of the muscarinic channel (Hill-Smith and Purves, 1978; Jalife and Moe, 1979a; Osterrieder et al., 1980), reaches a maximum (ΔV_{max}) and induces changes in the timing of the subsequent pacemaker discharge. The total duration of this hyperpolarization, the cophaseness (θ), is a function of the duration of VT (see Table 1) and is measured to the peak of the subsequent pacemaker discharge. The duration of the new period (T) resulting from the vagal input is determined by the relationship

$$T = \theta + (\phi + L). \quad (1)$$

Since the changes induced by a single vagal stimulus are short lived and the pacemaker rapidly (5–10 seconds) returns to its free running cycle (Jalife and Moe, 1979a), the delaying or accelerating effects can be described as phase shifts ($\Delta\phi$) of the pacemaker period with respect to its original rhythm (Fig. 1). The phase shift is determined by the relationship

$$\Delta\phi = T - \tau_{FR}. \quad (2)$$

The phase-response curve (PRC) is a plot of the phase shift induced by the vagal input as a function of the phase at the moment of the input.

Entrainment of the pacemaker occurs when its period is equal, or harmonically related, to the period of the vagal input, although not necessarily with a stable phase relationship. In contrast, synchronization is entrainment with exactly simultaneous pacemaker and vagal discharges (Aschoff, 1965). Nevertheless, in this article, the terms entrainment and synchronization will sometimes be used as synonyms.

Experimental Procedures

Isolated sinus node preparations were excised from 13 albino rabbits (1.5–3.0 kg) anesthetized by intravenous in-

TABLE 1
Phasic Effects of Brief Vagal Input on Pacemaker Cells from Nine Rabbit and Five Cat Sinus Node Preparations

	AMP (mV)	MDP (mV)	τ_{FR} (msec)	ϕ_{max} (msec)	ϕ_{max} (% τ_{FR})	L		ΔV_{max} (mV)		
						msec	% τ_{FR}	50	100	150
Rabbit SA node	58.4 \pm 2.5 (18)	-54.6 \pm 1.64 (18)	356.4 \pm 10.3 (18)	186.7 \pm 8.4 (18)	52.7 \pm 1.8 (18)	80.8 \pm 3.6 (18)	23 \pm 1.3 (18)	10.6 \pm 2.2 (15)	16.5 \pm 2.0 (16)	21.8 \pm 2.3 (13)
Cat SA node	74.8 \pm 4.2 (9)	-65 \pm 2.8 (9)	610.6 \pm 13.1 (9)	324.1 \pm 26.6 (9)	53.5 \pm 5.1 (9)	106.7 \pm 8.2 (9)	17.6 \pm 1.5 (9)	5.6 \pm 1.4 (8)	7.9 \pm 1.4 (8)	10.1 \pm 1.5 (7)
	$\Delta\phi_{max}$ (msec)			$\Delta\phi_{max}$ (% τ_{FR})			θ (msec)*			
	50	100	150	50	100	150	50	100	150	
Rabbit SA node	312 \pm 37.0 (15)	379.4 \pm 37.9 (16)	500.8 \pm 38.8 (13)	84.6 \pm 9.3 (15)	105.0 \pm 10.1 (16)	137.8 \pm 10.4 (13)	401.3 \pm 35.6 (15)	470.6 \pm 35.7 (16)	590.4 \pm 36.8 (13)	
Cat SA node	225.8 \pm 39.3 (8)	279.3 \pm 43.0 (8)	314.9 \pm 63.6 (7)	38.2 \pm 7.3 (8)	47.1 \pm 7.8 (8)	51.4 \pm 10.2 (7)	406.1 \pm 25.6 (8)	459.6 \pm 33.6 (8)	493.6 \pm 55.5 (7)	

All values are expressed as mean ± SEM. Data were obtained from one or more single train PRC's in each experiment at three different train durations (50, 100, and 150 msec). AMP: action potential amplitude; MDP: maximum diastolic potential; τ_{FR} : free-running period; ϕ_{max} : phase of maximum vagal effect (in msec and as percent of τ_{FR} ; L: latency to hyperpolarization (see Fig. 1); ΔV_{max} : maximum change in membrane potential; $\Delta\phi_{max}$: maximum delaying effect; θ : cophaseness, measured from onset of hyperpolarization to peak of delayed pacemaker discharge. Numbers in parentheses correspond to number of determinations of a given parameter.

* Statistical analysis by a "fitting constants" method (Steel and Torrie, 1980) demonstrated no significant difference between cat and rabbit θ in response to a given vagal train duration.

jection of sodium pentobarbital (35 mg/kg). Preparations were also obtained from seven young cats and one sheep. The chest was opened through a midline incision, and the heart was excised and placed in warm oxygenated Tyrode's solution.

Small rectangular blocks (1.0 cm²) of right atrial tissue containing the sinoatrial region were excised. Spontaneously beating preparations were transferred to a Plexiglas tissue bath and pinned to the wax bottom of the chamber with the endocardial surface up. Equilibration was allowed for at least 1 hour while superfusing the preparation at a rate of 20 ml/min with a modified Tyrode's solution that was saturated with a mixture of 95% oxygen and 5% CO₂. Unless otherwise indicated, temperature was maintained at 36–37°C. The composition of the solution (mM) was NaCl, 130; KCl, 4; NaH₂PO₄, 1.2; NaHCO₃, 24; CaCl₂, 1.8; MgCl₂, 1.0; and glucose, 5.6.

Transmembrane potentials were recorded from the sinoatrial pacemaker region with microelectrodes filled with 2.7 M KCl and connected to high input impedance amplifiers (WPI models 750 and KS – 700). The "true" pacemaker region was identified by the action potential morphology and by locating the site of earliest activation with respect to an external surface recording obtained from the crista terminalis.

Dynamic vago-nodal interactions were studied under conditions in which postganglionic vagal nerve terminals were stimulated following a technique modified from Vincenzi and West (1963). Electrical stimuli were applied through a pair of silver electrodes separated by 3–5 mm and positioned directly on the endocardial surface of the cephalic portion of the node. Stimuli were generated by a P6i Frederick Haer stimulator and usually consisted of brief trains (50–100 msec) of biphasic pulses, 50–150 μ sec in duration, 10–20 V in amplitude, and frequency of 200 Hz. These trains were subthreshold for sinoatrial node cells but were of sufficient magnitude to stimulate postganglionic vagal nerve terminals. No qualitative differences were found in the vagal effects induced by postganglionic stimulation in these small preparations and those observed in previous experiments with preganglionic vagal nerve stimulation (Jalife and Moe, 1979a).

In all experiments, activation of β -adrenergic receptors was prevented by constantly superfusing the preparation with Tyrode's solution containing propranolol 3.9×10^{-6} M (1 μ g/ml). After equilibration under these conditions, the magnitude of the responses to vagal stimulation was constant and remained stable for the duration of the experiment (6 hours or longer).

Each experiment consisted of three consecutive parts. First, the phasic effects of brief vagal discharges on sinoatrial pacemaker activity were assessed by applying single vagal trains every 10–20 seconds, scanning the spontaneous pacemaker period. The upstroke of the action potential was used to trigger the stimulator after variable delay. In some experiments (see Figs. 3 and 4) a second P6i stimulator was used to deliver slow frequency background stimulation through the same pair of surface electrodes. This enabled us to control the spontaneous pacemaker frequency and to determine the phasic effects of brief vagal input at various pacemaker cycle lengths in the same preparation.

In the second stage, brief vagal stimuli were applied after every pacemaker discharge (phase coupling; Levy et al., 1969). In this manner, steady state phase response curves were obtained by varying the position of the vagal stimulus in steps of 20–50 msec, over the entire pacemaker period. The PRC's obtained for single and for steady state condi-

tions were compared in terms of shape, duration of latent period, and inhibitory effects, as well as in terms of peak delaying and acceleratory components. These comparisons aided us in making quantitative predictions about more complex interactions during repetitive vagal discharges.

The predictive value of the single train and steady state PRC's was tested in the final experimental stage during which rhythmic vagal stimulation was applied at periods that were independent of the sinoatrial pacemaker rhythm (entrainment paradigm). The steady state interactions between the periodic vagal input and the pacemaker cycle length were determined at vagal stimulus periods that ranged between 200 and 1200 msec, in steps of 20–50 msec. At each vagal period, pacemaker cycles were recorded until a stable pattern of rhythm was established. Then vagal stimulation at a new cycle length was applied. The free-running pacemaker period was recorded before and after each completed run.

Statistical Analysis

Statistical comparison of cophase function (θ) obtained in rabbit and cat experiments (Table 1) was made using the method of "fitting constants" (Steel and Torrie, 1980), a randomized block design assuming no interactions. The data in Table 2 were studied via analysis of variance (ANOVA) using a randomized block design (Wallenstein et al., 1980), subsequent to which orthogonal comparisons were constructed to partition the treatment sum of squares into its component parts (Steel and Torrie, 1980).

Results

Effects of Single Vagal Perturbations

Brief vagal stimuli induce phasic changes in pacemaker periodicity that result from transient hyperpolarizations of the sinoatrial nodal cell membrane during the first and sometimes the second cycle after the vagal burst. Previous experiments from our laboratory have provided indirect evidence for the cellular mechanisms of these changes, and have given important insight about the dynamic interactions that may take place when the sinoatrial pacemaker becomes synchronized to the repetitive efferent vagal discharges. In this study, a more direct approach has been used to determine how the sinoatrial pacemaker responds to single and repetitive vagal input, and to provide a more quantitative account of the relationship between the pacemaker response to a single perturbation and its entrainability by the vagal discharge (Jalife and Moe, 1979a).

Examples of vagally mediated phase-dependent effects are illustrated in Figure 2, obtained from an isolated rabbit sinoatrial node. In each panel (A–C), two superimposed transmembrane potentials from a pacemaker cell are shown under control conditions, and during the application of a brief (50 msec) postganglionic vagal train of supramaximal intensity at three different phases of the pacemaker period. The horizontal bars show the timing of the vagal stimulus. The same impalement was maintained throughout. In all panels, the pacemaker was beating at a free-running period of about 335 msec. Panel A shows the effects of a vagal input applied at an early phase.

TABLE 2
Single vs. Repetitive Phase Coupled Vagal Train Application for Predicting Entrainment Behavior of Rabbit Sinus Node Pacemakers

Experiment date	τ_{FR}	Single train* ($\Delta\phi_{max}$)			Phase-coupled trains ($\Delta\phi_{max}$)			Maximum limits of entrainment (T_{max})		
		50 msec	100 msec	150 msec	50 msec	100 msec	150 msec	50 msec	100 msec	150 msec
1/14/82	294.84±1.5 (30)	99	113	200	91	95	101	81	81	132
1/28/82	356.1±2.2 (33)	130	206	224	45	42	56	27	40	40
2/17/82	356±1.1 (30)	108	112	110	49	56	77	39	25	51
4/06/82	349±0.69	86	110	124	38	70	73	32	58	73
		105.8±10.7†	135.3±27.3	164.5±32.2	55.8±13.8	65.8±13.0	76.8±10.7	44.8±15.8	51±13.9	74±23.7

* Orthogonal comparisons indicated that single train results were significantly different from phase-coupled and entrainment data ($F = 50.03$, $P < 0.005$). No difference was found between phase-coupled and entrainment results. τ_{FR} = mean free-running period; $\Delta\phi_{max}$ = maximum phase shift induced by vagal train at each duration expressed as % τ_{FR} . T_{max} is expressed as percent change from τ_{FR} . All data are positive and indicate prolongation of pacemaker period.

† Values are means ± SE. The initial analysis of variance for treatment effect produced an F value of 7.76 which was significant at the level $\alpha = 0.005$ ($P < 0.005$).

After a latency of about 70 msec, and as a result of acetylcholine-receptor interactions, the cell membrane was hyperpolarized and the next spontaneous discharge was delayed by 85 msec. In panel B, a similar train applied at a later phase (125 msec) induced a greater prolongation (195 msec) of the pacemaker period. Maximal prolongation was observed at a stimulus phase of 180 msec (panel C) which yielded a pacemaker period that was 225 msec (67.2%) longer than control. Panel D shows four superimposed recordings illustrating the strong relationship between vagal stimulus phase and the delaying effects on the pacemaker cycle. When the position of the vagal stimulus was shifted to later phases that were within the last 150 msec of the free-running period (not shown), there were no immediate effects on the pace-

maker rhythm, and the pacemaker discharge occurred on schedule. The hyperpolarizing and delaying effects were postponed to the following cycle.

These results are typical of all rabbit SA node experiments surveyed and are qualitatively similar to those obtained in other species (cat, dog, sheep). This is illustrated in Table 1, which shows composite data obtained in nine rabbit and five cat sinus node preparations for brief vagal trains of three different durations (50, 100, 150 msec). Observations were made for trains applied at the phase (ϕ_{max}) at which the vagally induced delay in pacemaker discharge ($\Delta\phi_{max}$) was maximal. There were large differences between the two species in terms of control action potential amplitude, maximum diastolic potential, and free-running pacemaker period. The difference in τ_{FR} resulted in a different absolute value of ϕ_{max} for each species. However, this difference disappeared when ϕ_{max} was normalized and expressed in terms of percent τ_{FR} . The most outstanding differences were found in the comparison of the maximal hyperpolarization (ΔV_{max}) and the $\Delta\phi_{max}$ induced by the respective vagal trains, thus suggesting that ΔV_{max} could be the most important determinant of the phase shift generated by a given vagal input. Yet, a closer look at Table 1 reveals that the duration of the hyperpolarizing action (ie., the cophasic, θ) at each individual train duration is almost the same in the two species, in spite of major differences in ΔV_{max} . The cat vs. rabbit data for the variable θ were analyzed via a computer program utilizing the method of "fitting constants" (Steel and Torrie, 1980), a randomized block design that assumes no interaction between animals and vagal pulse duration. This analysis yielded an $F = 1.82$ for the interaction term and an $F = 1.64$ for the effect of animal ignoring vagal train duration, both of which were not significant. Since this indicates a statistical similarity between cat and rabbit θ in response to a

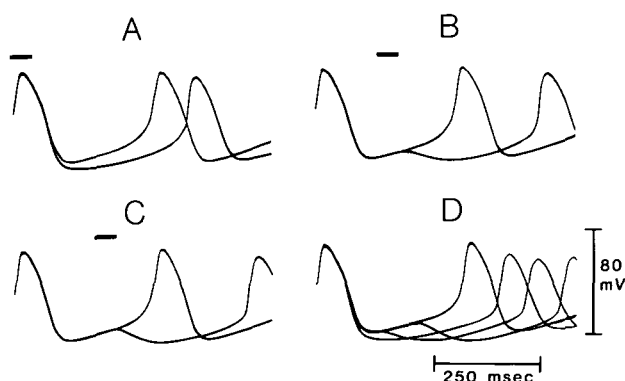


FIGURE 2. Phase-dependent effects of brief vagal stimulation on pacemaker periodicity of a rabbit sinoatrial nodal cell. Single vagal trains were applied postganglionically every 10 seconds. Each train consisted of 10 biphasic pulses, 100 μ sec in duration, 5 msec apart and supramaximal intensity. Same impalement in all panels. In panels A–C, control traces are superimposed with traces obtained during application of a vagal train (horizontal bar) at three different phases. In panel D, four superimposed traces are shown for comparison. Free-running period, 335 msec. Upstroke retouched.

given vagal train duration, θ may be considered a constant for the train duration in question. This indicates that, since τ_{FR} in the cat sinus node is almost twice as long as in the rabbit (Table 1), the magnitude of $\Delta\phi_{max}$ at ϕ_{max} is determined primarily by the difference between the duration of τ_{FR} and the duration of θ in such a way that

$$\Delta\phi_{max} = \tau_{FR} - (\theta + (\phi_{max} + L)) . \quad (3)$$

Effects of Change in Phase

The dependence of these effects on the characteristics of the vagal input and on its position within the pacemaker period are visualized in the graphic display of Figure 3. The data were obtained from a rabbit SA node preparation in which brief postganglionic vagal stimulation at two different train durations was used to scan the pacemaker period. In each panel, time zero (abscissa) corresponds to the beginning of the spontaneous cycle during which vagal stimulation was applied and whose scheduled termination is indicated by the thin vertical lines. The phase and duration of the vagal stimulus is represented by the horizontal bars scanning the free-running cycle (ordinate). The time of occurrence of the initial four pacemaker discharges after each stimulus is indicated on the abscissa by the respective symbols. In panel A, the mean free-running pacemaker period was 418 msec, and a 75-msec vagal train was used for the scan. At this vagal train duration, the maximum level of hyperpolarization at any stimulus phase was approximately constant (see also Fig. 2D). Thus, whenever the stimulus was applied at phases briefer than 300 msec (Fig. 3A),

the interval between the vagal train and the delayed pacemaker discharge was relatively fixed. At later phases, the relationship changed abruptly; the first pacemaker discharge was no longer delayed and the vagal effects were postponed to the second pacemaker cycle. Yet, a constant relationship in the interval between the stimulus and the delayed discharge (i.e., a constant cophase, θ) was maintained in such a way that the lines defining the positions of first (closed circles) and second (open triangles) discharges appeared to fuse into one.

The results shown in Figure 3A are representative of all experiments in which supramaximal trains of relatively long durations (50–150 msec) were applied either to the cervical vagus (Jalife and Moe, 1979a) or to the postganglionic vagal terminals in the subendocardium of the sinus node. At briefer vagal stimulus durations (Fig. 3, panel B) or at lower intensities, the amplitude of the hyperpolarization was no longer constant, the stimulus-to-pacemaker discharge interval decreased progressively, and the linearity of the phasic pacemaker response to the vagal stimulus disappeared.

One additional finding in the experiment of Figure 3 should be considered. It is clear from panels A and B that, regardless of the stimulus characteristics, in this rabbit SA node preparation, only the primary inhibitory component of the vagal effects (Brown and Eccles, 1934) is apparent during the first and second cycles after the vagal input. Since, after the second cycle, the pacemaker returned to its free-running period, in the subsequent cycles the effects are manifest as a phase shift of the spontaneous rhythm with

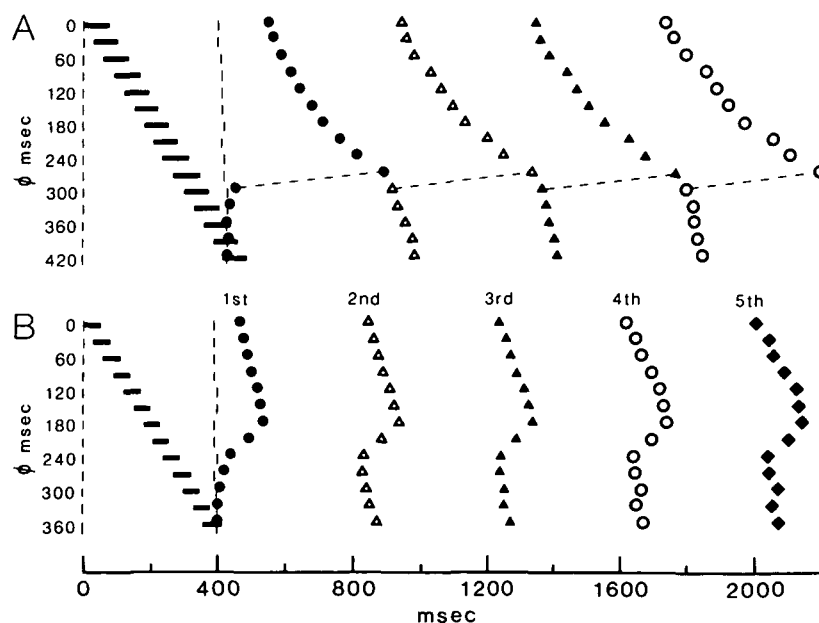


FIGURE 3. "Real-time" plots of vagal effects on spontaneous activity of a rabbit sinoatrial pacemaker at two different vagal train durations. In both panels, the vagal input phase (ϕ), expressed in milliseconds, is plotted on the ordinate scale. The changes in pacemaker cycle length during the initial five beats after the train are denoted by the respective symbols, and plotted on the abscissa. In panel A, 75-msec trains during the initial five beats after the train are denoted by the respective symbols, and plotted on the abscissa. In panel A, 75-msec trains (horizontal bars) were applied singly every 10 seconds scanning the entire pacemaker period, 30-msec steps. At $\phi < 300$ msec, large inhibitory effects on first (black dots) and second (open triangles) cycles phase shifted all subsequent discharges. At later phases, the first cycle was not affected but the vagal effects were manifest in subsequent periods. In panel B, 50-msec trains were used for the scan. Note that in this case a continuous nonlinear phase response relationship was maintained.

respect to the control. In the original report of Brown and Eccles (1934), two types of inhibitory curves were described. The first type showed a time course similar to that in Figure 3, with the vagal effects decaying very rapidly within the first or second pacemaker cycle. In the second type, a triphasic time course was observed in which the primary inhibitory component was followed by a phase of acceleration and a secondary inhibition that lasted several seconds. No acceleratory or secondary inhibitory effects are apparent in the experiment of Figure 3. This was commonly (not always) observed in rabbit SA node preparations, but was by no means the rule when cat sinus node was studied (see Fig. 6). Triphasic inhibitory curves were always obtained in cat preparations regardless of the method of stimulation (pre or postganglionic).

The lack of an acceleratory component, as we shall demonstrate below, may have been fortuitous (see Figs. 4 and 5). However, the reason for the absence of secondary inhibitory effects in some of these preparations is as yet unclear, since the subcellular basis of the decreased slope of phase 4 depolarization that leads to the secondary inhibition (Jalife and Moe, 1979a) has not, to our knowledge, been documented experimentally.

Paradoxical Effects; Can the Vagus Accelerate the Heart?

The data presented thus far demonstrate that the effects of brief vagal stimulation on the sinoatrial pacemaker period are dependent not only on the timing, amplitude, and duration of the vagally induced hyperpolarization, but also on the relationship between the duration of the hyperpolarization and the duration of the free-running pacemaker period (Table 1). Previous experiments from our laboratory (Jalife and Moe, 1979a) have suggested that every time a pacemaker cycle was scheduled to end slightly after the termination of the vagally induced hyperpolarization, a post-inhibitory rebound could, in fact, advance the subsequent pacemaker discharge and, paradoxically, abbreviate that cycle. However, no definite proof of this paradoxical acceleration was obtained at that time, and it was necessary to infer the mechanism of the phenomenon indirectly through evidence obtained during hyperpolarizing current pulse application in a sinus node-sucrose gap preparation (Jalife and Moe, 1979a).

A striking example of vagally induced pacemaker acceleration is illustrated in Figure 4, obtained from a rabbit SA node preparation. All panels show simultaneous transmembrane potentials recorded from a cell in the pacemaker region (top trace), and from a subsidiary pacemaker cell in the septal edge of the node (middle trace). The bottom trace is a septal surface recording and stimulus monitor. The temperature was held constant at 32°C; this served to prolong the free-running period. In addition, low frequency (6.8 Hz) background vagal stimulation was applied continuously to further prolong the pacemaker cycle

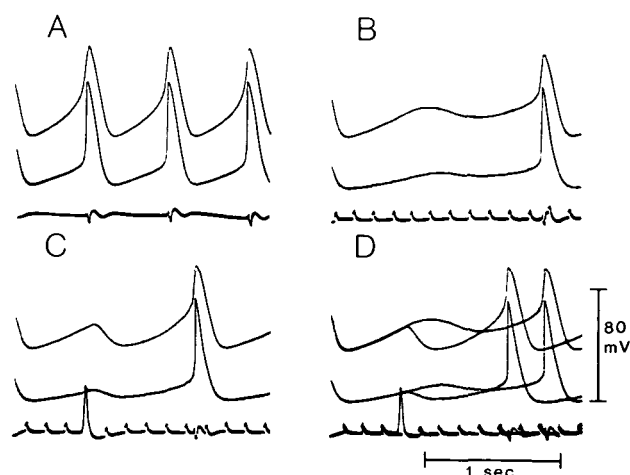


FIGURE 4. Vagally-induced acceleration of pacemaker activity in a rabbit SA node preparation. In all panels, simultaneous transmembrane potential recordings from "true" and subsidiary pacemaker cells are shown in top and middle traces, respectively. The bottom trace is a bipolar surface recording from the septal border of the node, and served as vagal stimulus monitor (see also horizontal bars in panels C and D, top). Propranolol (1 $\mu\text{g}/\text{ml}$) was present throughout; temperature was held constant at 32°C. Panel A, free-running period (590 msec) in the absence of vagal stimulation. In panel B, postganglionic stimulation at a low frequency (6.8 Hz) prolonged the pacemaker period. In panel C, a brief, high frequency (200 Hz), vagal train was applied during continuous background stimulation. No pacemaker shifts were apparent. However, note hyperpolarization and rapid "rebound" of membrane potential. The pacemaker period was abbreviated by 270 msec. In panel D, two superimposed sweeps are shown for comparison. Same impalement in all panels. Upstrokes retouched.

(panels B–D). Furthermore, propranolol was continuously superfused at a concentration of 1 $\mu\text{g}/\text{ml}$. Panel A shows the steady state free-running pacemaker period (590 msec) at 32°C in the absence of vagal stimulation. In all panels, the oscilloscope was triggered by the action potential downstroke. In panels B–D, recordings were obtained during the diastolic interval and subsequent pacemaker discharge which occurred at a cycle length of about 1690 msec (panel B) after a subthreshold oscillation. This oscillatory behavior is common of sinoatrial pacemakers in response to long-lasting hyperpolarizations (Noma and Irisawa, 1974) and is predicted by theoretical calculations (Michaels and Jalife, unpublished), using Hodgkin and Huxley-type equations (Yanagihara et al., 1980). In panel C, a brief (50 msec) high frequency train (200 Hz) was applied at a phase of 630 msec. After a 100-msec latency, the pacemaker cell membrane (top trace) hyperpolarized almost to the level of the maximum diastolic potential. However, this hyperpolarization was transient ($\theta = 690$ msec), and the membrane depolarized rapidly toward threshold, discharging an action potential at a cycle length (1420 msec) that was 270 msec briefer than in B. Note that—in spite of this significant abbreviation—the activation sequence remained unchanged, and no shift of pacemaker site was apparent in the subsidiary pacemaker or the septal recording. Two

oscilloscope sweeps are superimposed in panel D to compare "control" and test responses. Clearly, brief vagal stimulation at supramaximal intensity can significantly shorten the pacemaker cycle length under the appropriate conditions; i.e., when the pacemaker period is sufficiently long to outlast the resultant hyperpolarization. As demonstrated by the traces in Figure 4, this abbreviation is secondary to the hyperpolarization and subsequent "rebound" of the pacemaker cell membrane and does not depend on any external factors, such as pacemaker shifts or reentrant mechanisms (Spear et al., 1979).

Additional demonstration of this phenomenon is given in Figure 5. The conditions in this rabbit SA node experiment were the same as those in Figure 4, except that the temperature was maintained at 37°C. The duration of the pacemaker period was controlled by applying background postganglionic stimulation at various frequencies. Two superimposed traces are shown in each panel of Figure 5 to compare the pacemaker period in the absence and the presence of a brief (50 msec), high frequency vagal stimulus applied at a fixed interval (top trace). The same impalement was maintained throughout. In panel A, the free-running (no background stimulation) pacemaker period was 405 msec. A brief vagal input applied very early in the cycle hyperpolarized the membrane and delayed the next pacemaker discharge by 170 msec. In panel B, continuous low frequency (5 Hz) back-

ground stimulation increased the pacemaker period to 600 msec. Application of the test vagal stimulus further prolonged the pacemaker period, but in this case the relative increment in cycle duration was much less (48 msec) than in panel A. Note that following the hyperpolarization induced by the brief train, the slope of phase 4 depolarization was steeper than during the respective control (panel B), especially at the end of the diastolic interval. In panel C, the background frequency was increased to 10 Hz, and the pacemaker period was prolonged to 690 msec. In the following sweep, the brief train once again was followed by a membrane hyperpolarization. In this case, however, the steeper diastolic slope effectively accelerated the subsequent pacemaker discharge in spite of continuous background vagal stimulation. Finally, in panel D, stimulation at 15 Hz prolonged the pacemaker period to 775 msec. Under these conditions, the acceleratory effects after the brief stimulus became more pronounced and the pacemaker cycle length was abbreviated by about 120 msec.

It should be pointed out that, in Figures 2, 4, and 5, the test responses, whether delayed or accelerated, were of lesser amplitude and duration than their respective controls. This probably resulted from a moderate inhibitory action of acetylcholine on the slow inward current responsible for the action potential upstroke in these cells (Brown et al., 1979). However, it should also be indicated that this effect plays a very minor role in determining the phasic nature of the changes induced by the brief vagal input on pacemaker periodicity. Indeed, the results of these experiments show that for a given vagal stimulus (see Figs. 2-5), the absolute magnitude of the phase shift (whether delay or acceleration) is a function of the stimulus timing, of the duration of the hyperpolarization, and of the duration of the free-running pacemaker period (see also Table 1).

The PRC; Single vs. Repetitive Input

The phase response curve is a convenient way to illustrate the phasic nature of the response of an oscillatory system to brief perturbations from its surroundings (Pittendrigh, 1965; Jalife and Moe, 1979b; Jalife et al., 1980a). Two types of PRC's are generally used in cardiac electrophysiology to describe the effects of brief vagal perturbations. In the procedure illustrated in Figures 2 and 3, the pacemaker period is scanned by applying a single vagal stimulus every 10-30 second. Data may be plotted as "inhibitory curves" (Brown and Eccles, 1934) which show the effect of the single vagal stimulus on several cardiac cycles (Fig. 6A), or as "PRC's" which depict only the effect on the pacemaker discharge immediately following the stimulus. In the second type of experiment, perturbations are applied periodically at fixed intervals after each pacemaker discharge. The steady state effect on pacemaker period is then plotted on the PRC as a function of the phase of the vagal stimulus (Levy et al., 1969). This protocol is referred to as phase coupling.

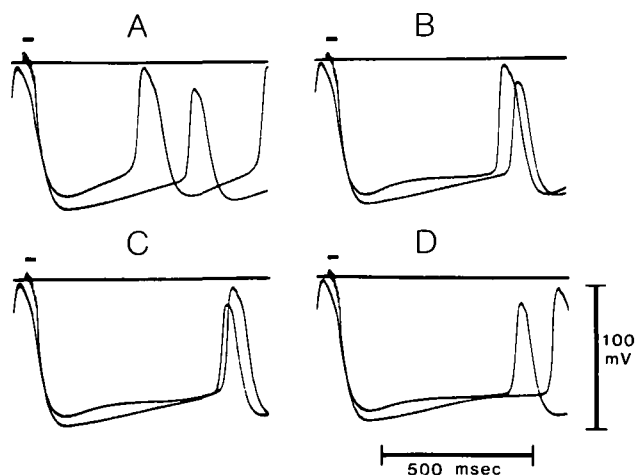


FIGURE 5. Cycle-length dependence of vagally induced pacemaker acceleration. Rabbit SA node superfused with Tyrode's containing propranolol (1 μ g/ml) at 37°C. Top trace, brief train monitor; bottom trace, transmembrane potential recordings. Two superimposed traces are shown in each panel. Panel A shows the control (i.e., no background stimulation); a brief (50 msec) vagal train applied at $\phi = 40$ msec hyperpolarized the membrane and prolonged the pacemaker period from 405 to 575 msec. In panels B, C, and D, the duration of the pacemaker period was controlled at will by continuous background stimulation of postganglionic fibers at 5, 10, and 15 Hz, respectively. Depending on the duration of the corresponding control, test vagal trains applied every 10 seconds at constant ϕ (i.e., 40 msec) yielded either slight prolongation (panel B), slight abbreviation (panel C), or large shortening (panel D) of the pacemaker period. Upstrokes retouched.

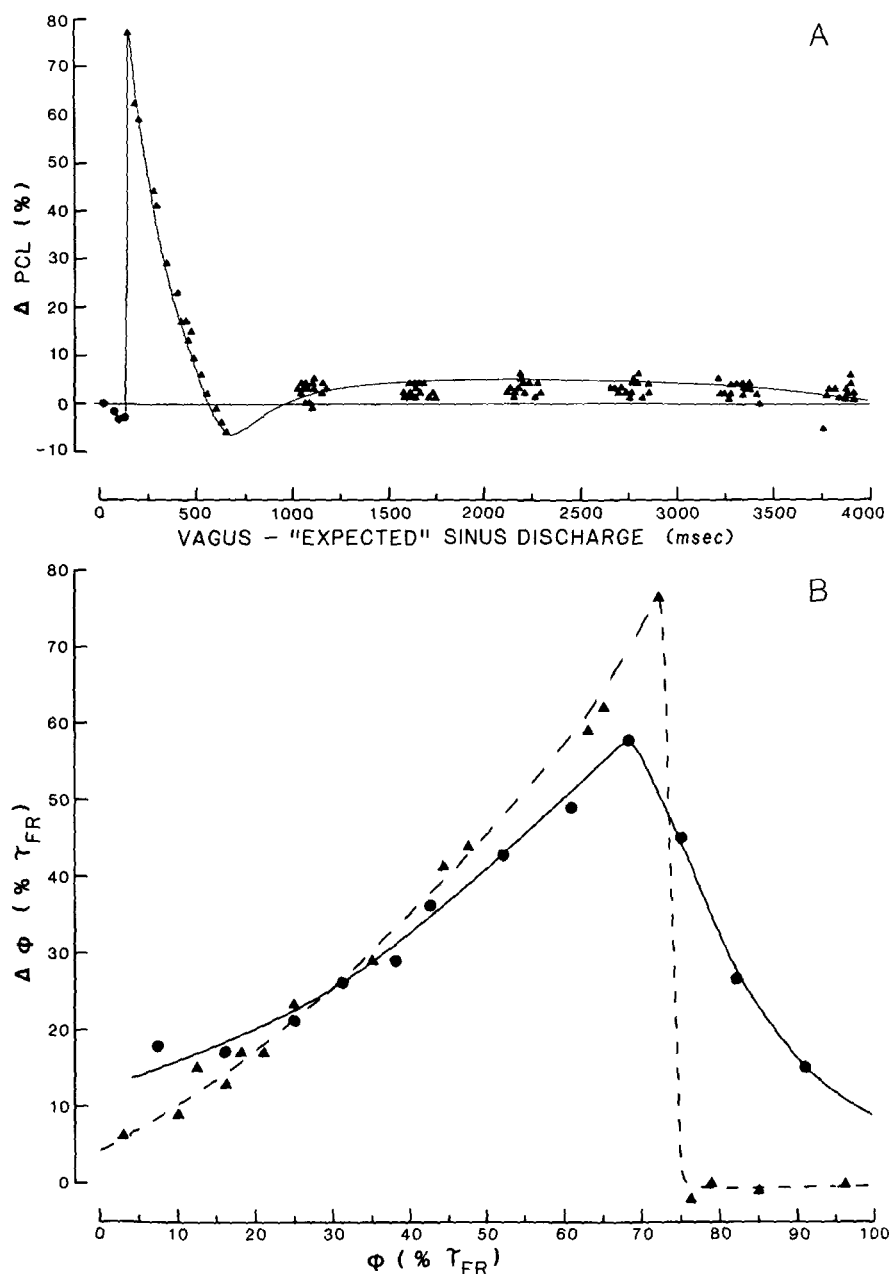


FIGURE 6. Panel A, triphasic effects of brief vagal stimuli scanning the spontaneous pacemaker cycle of a cat sinus node preparation. Brief trains (100 msec) were applied postganglionically every 10 seconds. Ordinate, change in pacemaker cycle length (ΔPCL), expressed as percent of free-running period (570 msec), denoted by the thin horizontal line. Abscissa, interval between onset of vagal input and subsequent ("expected") sinoatrial pacemaker discharge. The black dots indicate the position of first beat when vagal input was too late to affect the first pacemaker cycle [i.e., the "latent period" of Brown and Eccles (1934)]. The black triangles show the effects on the initial seven pacemaker cycles during the primary inhibitory and acceleratory, as well as the secondary inhibitory effect. Panel B, phase response curves (PRC's) obtained in same experiment as in panel A, and measured during the first cycle of impinging stimulus. The data of single train (black triangles) and repetitive ("phase-coupled"; black dots) vagal stimulation are expressed on the ordinate as phase shifts ($\Delta \phi$'s) in terms of percent of the free-running period (τ_{FR}) vs. the phase (ϕ) of the vagal input also expressed as percent of τ_{FR} . See text for further details.

Figure 6 compares the results of these two methods in a cat SA node experiment. Panel A shows the effect of single vagal trains (100 msec), plotted as an inhibitory curve (Jalife and Moe, 1979a) modified from the original Brown and Eccles (1934) plot. The percent change in pacemaker cycle length (PCL) of the initial seven beats after the stimulus is plotted on the ordi-

nate scale as a function of the interval between the stimulus and the expected pacemaker discharge. A triphasic inhibitory curve resulted. Beginning at time zero on the abscissa, there was a latent period of about 135 msec, a phase of prolongation of the first pacemaker cycle, an acceleratory component during the second pacemaker cycle, and a final phase of

lesser deceleration that lasted for several seconds. In panel B, the data for the first cycle (black triangles) are replotted in a PRC ($\Delta\phi$ vs. ϕ) and are compared with data obtained using phase coupled stimulation (black dots). As the superimposed curves in Figure 6B show, vagal inputs can lead to strong phase-dependent effects, regardless of the method of stimulus application. In either case, stimuli applied progressively later in the cycle induce progressively greater prolongation in pacemaker period. The two curves show some differences in general shape and extreme values. There are two main reasons for these differences. First, in the single train PRC, only the effects on the first pacemaker discharge after the stimulus are taken into account. Thus, the residual effects during the second cycle and secondary inhibitory components of the subsequent cycles (panel A) are lacking. In contrast, during phase-coupled stimulation, even though in the steady state the phasic nature of the pacemaker response to the vagal input remains unaltered, the buildup of secondary components over several cycles contributes to a shift in the baseline toward longer pacemaker periods and, consequently, to a shift in the magnitude of the individual responses.

The second and perhaps more important reason for the quantitative differences between single train and steady state PRC's is the development of desensitization of the muscarinic receptor at the sinoatrial cell membrane (Jalife et al., 1980b; Tokimasa, et al., 1980). A decay of the chronotropic response of the sinoatrial pacemaker usually develops after long periods of vagal stimulation at relatively high frequencies (Sawto, 1968; Jalife et al., 1980b; Martin et al., 1982). This fade has been ascribed to receptor desensitization or possibly to a combination of receptor and effector desensitization (Martin et al., 1982). It follows that, with repetitive vagal input, the steady state PRC frequently is reduced in amplitude because the hyperpolarizing effect of the individual trains is ameliorated, especially at the faster frequencies.

Entrainment and the PRC

One of the most outstanding features of the dynamic control of heart rate by parasympathetic activity is the ability of periodic vagal discharges to entrain the pacemaker to beat at periods that may be briefer or longer than its own intrinsic cycle length (Suga and Oshima, 1969; Reid, 1969; Levy et al., 1969; Jalife et al., 1982). The possibility of entrainment depends mainly on the fact that brief vagal discharges can "correct" the pacemaker rhythm by an amount that depends on their instantaneous relationship to the pacemaker period. Accordingly, since there is only one PRC for a particular stimulus under a specific set of circumstances, the PRC should provide quantitative testable predictions about characteristics of entrainment under those specific conditions.

The predictive value of the PRC was tested in 12 experiments. After the single and steady state PRC's had been completed, the entrainment paradigm was carried out (see Methods). Composite results of a

complete rabbit SA node experiment are illustrated in Figure 7. Each vagal stimulus consisted of a 50-msec train (10 pulses). Panel A shows single train (black triangles) and steady state (black dots) phase response curves. Panel B shows plotted results of the entrainment paradigm. The mean pacemaker period (T), expressed as a percent change in the free-running period (τ_{FR}), is plotted on the ordinate scale of Figure 7B, as a function of the vagal stimulus period (abscissa), expressed in msec. The horizontal broken line indicates no change in the free-running pacemaker period (380 msec); any deviation from this line is a measure of the extent of entrainment. All data were obtained during steady state conditions at each vagal stimulus cycle. The stable entrainment zones are identified by the inclines, and their slopes define the harmonic relations between the sinoatrial pacemaker and the repetitive vagal input.

Several predictions of the PRC were tested in this experiment. First, the strong phase sensitivity of the pacemaker period to the vagal input (fig. 7A) predicts that, during repetitive stimulation, it should be possible to entrain the pacemaker to beat at regular periods other than its own intrinsic cycle. This was clearly shown to be the case. Stable entrainment occurred at vagal stimulus to pacemaker cycle length (VSCL:PCL) ratios of 2:1, 1:1, and 1:2 (Fig. 7B). Within these zones, the pacemaker adopted a fixed phase relation to the vagal input and a constant discharge pattern at any vagal cycle length after a short "accommodation" period. The stable zones were separated by areas of instability in which wide variations in pacemaker cycle and phase relations occurred. Phase-fluctuating rhythmic patterns converted almost immediately when a stable zone of entrainment in the cycle length-response curve was reached.

A good experimental example of entrainment is shown in Figure 8. The free-running cycle length was 250 msec (panel A) and entrainment was attempted with vagal trains (vertical bars) that greatly delayed the pacemaker discharge. In panels B-D, stable entrainment could be maintained at periods (480, 500, and 520 msec) that were approximately twice as long as the free-running period. At longer cycles (not shown), entrainment was broken until the vagal period was increased to 700 msec (panel E), at which time the pacemaker was locked to beat in a 1:2 ratio. This experiment shows very clearly that the entrainment characteristics of the pacemaker to a given perturbation are dictated by the phasic relations between the pacemaker period and the period of the perturbing input.

The PRC and the Limits of Entrainment

The second important prediction of the PRC concerns the limits of entrainment of the pacemaker by the external periodicity. The results in Figure 7B indicate that stable entrainment can be maintained as long as the vagal cycle length is not too different from the free-running period or one of its harmonics. According to oscillator theory, the extreme values to

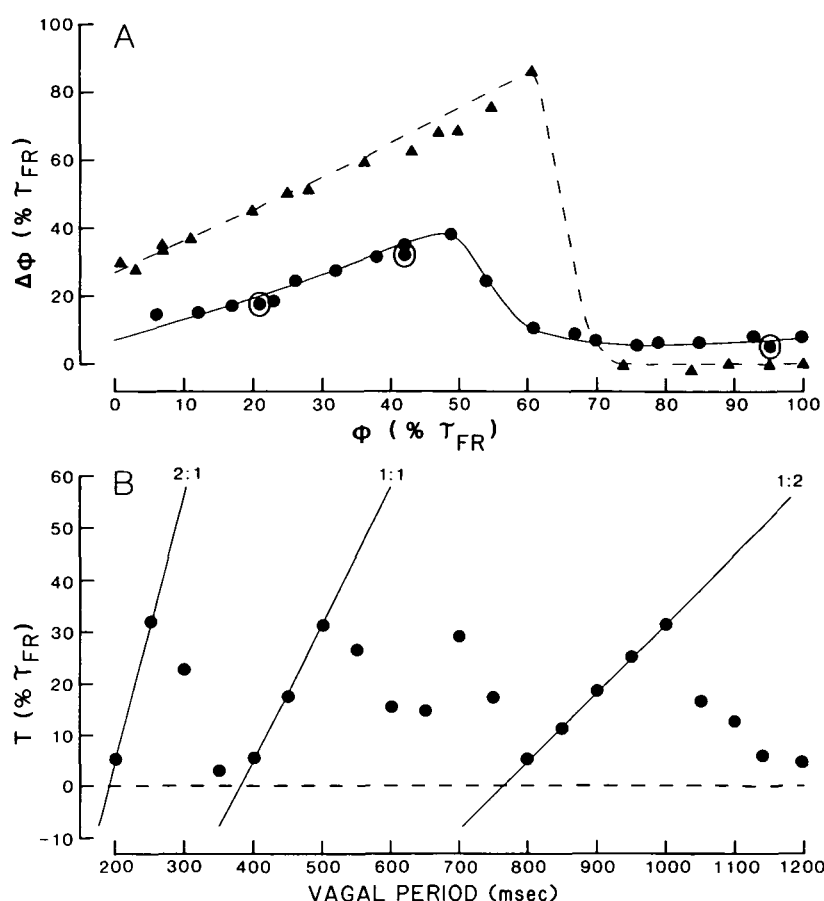


FIGURE 7. Test of accuracy of single train vs. steady state (phase-coupled) PRC's in predicting the entrainment behavior of the pacemaker in response to vagal input of intermediate strength. Panel A, experimental PRC's obtained from a rabbit SA node preparation. The free-running period (τ_{FR}) was 380 msec, and brief (50 msec) postganglionic trains were applied either singly (black triangles) every 10 msec or repetitively (black dots) after every pacemaker discharge scanning the pacemaker period. Vagally induced phase shift ($\Delta\phi$) is plotted as a function of the phase (ϕ) of the vagal train, both expressed as percent of τ_{FR} . Encircled dots represent data obtained during 1:1 synchronization in entrainment paradigm. Panel B, entrainment curve of same preparation during repetitive vagal stimulation at periods that were independent of τ_{FR} . Ordinate, entrained pacemaker period (T) expressed as percent change in τ_{FR} and as a function of the vagal period. Broken horizontal line shows lack of entrainment. Solid inclines represent zones of stable entrainment at harmonic vagus-sinus relations. Only 2:1, 1:1 and 1:2 are shown.

which a system will entrain are given by the extreme phase-shift that a single input (comparable to a recurring entraining input) will effect. If this phase shift is very large—and it can be more than twice the free-running period (see Fig. 8)—entrainment should occur at cycle lengths that are significantly different from the intrinsic pacemaker period. In Figure 7B, the limits of the zone of stable entrainment were clearly dictated by these phasic relations and were easily determined from the steady state PRC in Figure 7A. The extent of entrainment in this case (panel B) was always between 400 and 500 msec; i.e., between 5 and 32% longer than the free-running period (380 msec). These extreme values were very similar to those in the steady state PRC of panel A (5 and 38%). The slight difference (6%) at the higher limit resulted probably because, in the entrainment experiment, the vagal cycle length was varied in 50 msec steps, and some data points were omitted. In spite of this, the entrainment data (indicated by the encircled dots in Fig. 7A) show a

remarkably good correlation to the steady state PRC. This correlation was confirmed in four preparations (Table 2) in which the three consecutive paradigms (i.e., single train, steady state phase-coupling, and entrainment) were performed while recording sinoatrial pacemaker activity from the same pacemaker cell throughout each experiment. Analysis of variance, using a randomized block design (Wallenstein et al., 1980) and orthogonal comparisons, demonstrated no significant differences between the $\Delta\phi_{max}$ and maximum limit of entrainment values at the three different train durations (50, 100, and 150 msec). In contrast, a highly significant difference ($P < 0.005$) was found when the single train results were compared with the steady state and entrainment data. This difference cannot be related to a depletion of acetylcholine stores (Kilbinger and Löffelholz, 1976) at the nerve terminals since single train curves (not shown) obtained immediately after the entrainment paradigm, were nearly identical to those reported in Table 2.

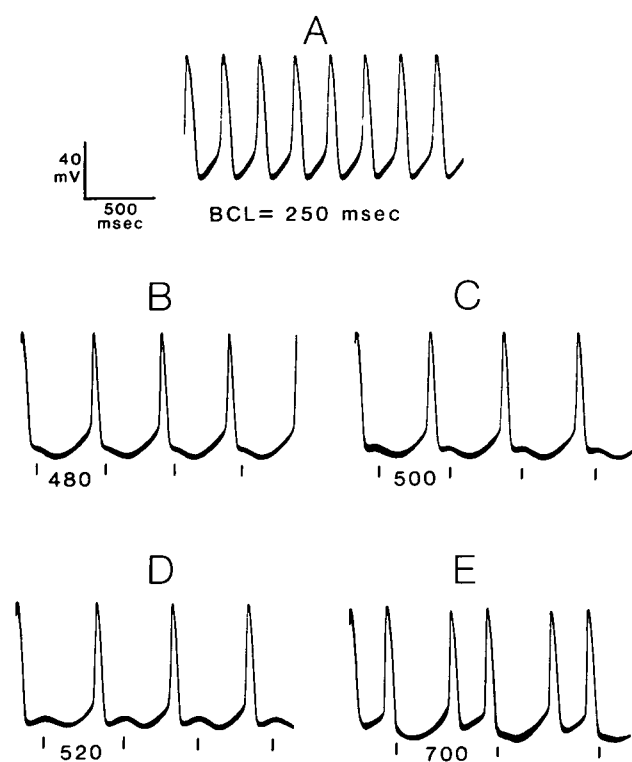


FIGURE 8. Analog records illustrating entrainment behavior of sinoatrial pacemaker preparation during application of repetitive vagal input. Panel A is the control. In panels B–D, brief vagal trains (vertical bars) were applied at periods of 480, 500, and 520 msec. 1:1 entrainment occurred, and the pacemaker was forced to beat at constant cycles that were about twice as long as the free running period. In panel E, at a vagal period of 700 msec, 2:1 entrainment ensued.

The Steady State PRC and The Limits of Entrainment

Although the single train PRC provides some important information about the entrainment behavior of the sinoatrial pacemaker, the steady state PRC is a much more quantitative indicator for predicting entrainment characteristics, especially when desensitization of cholinergic receptors alters the response of the pacemaker to periodic vagal input. Nevertheless, the PRC obtained in the single train experiment (Fig. 7A; black triangles) indicates that, in the absence of desensitization or when the steady state magnitude of the vagal input is increased, the limits of stable entrainment should become greater. This is precisely what happens (see Table 2). Figure 9 shows the steady state PRC (panel A) and entrainment curve (panel B) obtained from the same rabbit SA node experiment as in Figure 7, when the duration of the vagal train was increased from 50 msec (10 pulses) to 150 msec (30 pulses). Figure 9A shows that scanning the pacemaker cycle with these stronger inputs produced much greater delays in the steady state, up to a maximum of 71%, whenever the train was programmed to occur at a steady state phase of 56% of the free-running period. In panel B, when the vagal input was presented at periods that were independent

of the pacemaker discharge, the ranges of vagal cycle lengths at which the pacemaker could be entrained became wider (compared with Fig. 7B), at the expense of the zones of instability. The maximum limit of the stable 1:1 entrainment increased to a period of 650 msec, which corresponds to a maximum delay of about 71% (Fig. 9B).

The Stability of Entrainment

The PRC also predicts the phasic relations at which stable entrainment will occur. In Figure 7B, stable 1:1 entrainment occurred at vagal periods between 400 and 500 msec. The three points that comprise this region of stability correspond to repetitive vagal trains that occurred at fixed phases in the pacemaker period. These points fell on the positive slope regions of the steady state PRC (encircled dots; Fig. 7A). This was also true for the stable points in the 1:1 region of Fig. 9B. Outside of those regions (i.e., between 50 and 80% of the pacemaker period in Fig. 7B), phasic relations were unstable, fixed entrainment could not be maintained, and large beat-by-beat variations in pacemaker cycle length resulted. These data are in complete agreement with those of Levy and his co-workers (1969a) in the anesthetized dog, and conform to the mathematical predictions (Perkel et al., 1964) that the domain for stable entrainment should lie in those regions of the PRC having a slope between 0 and 2.

PRC Symmetry Determines the Lower Limit of Entrainment

The aforementioned PRC's (Figs. 7 and 9) were asymmetrical in the sense that vagal inputs induced delays at all phases in the cycle. The existence of this asymmetry predicted that stable entrainment would be limited to mean intervals that were longer than the free-running pacemaker period. However, the data of Figures 4–6, together with PRC's constructed in an earlier study (Jalife and Moe, 1979a), indicate that, under appropriate conditions, brief vagal inputs applied sufficiently early in the pacemaker period can accelerate the subsequent discharge. According to those results, the symmetry of the PRC depends very strongly on the relationship between the duration of the vagally induced hyperpolarization (i.e., θ) and the duration of the free-running period (see Table 1). Consequently, the ability of the vagal input to phase advance the pacemaker rhythm in those cases in which the intrinsic period is relatively long, predicts that it would be possible to entrain the pacemaker at intervals that are briefer than its free-running period.

Examples of vagally induced acceleration of pacemaker rhythm (Fig. 10) were obtained from an isolated sheep sinus node preparation that was beating at a slow pacemaker cycle length. The top panels show superimposed traces during control and during application of a brief vagal train. The mean free-running period was 1630 msec. In panel A₁, a brief vagal stimulus (horizontal bar), applied at a phase of 180 msec, advanced the next discharge and abbrevi-

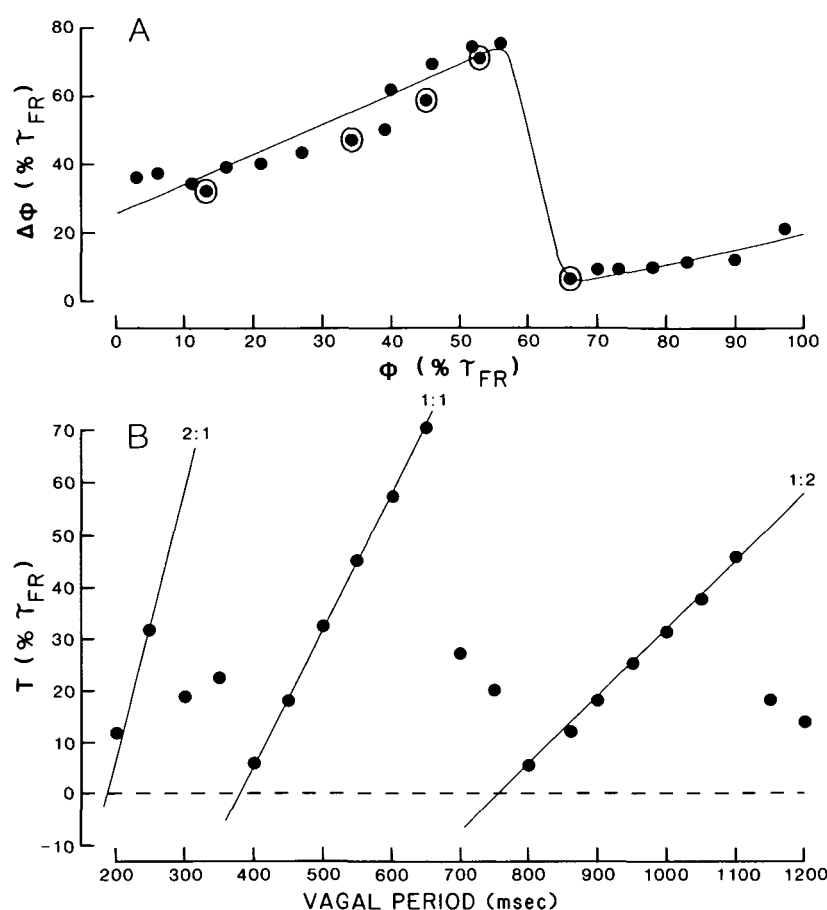


FIGURE 9. Entrainment characteristics in response to "strong" vagal input. Same experiment as in Figure 7. Panel A, steady state PRC (phase coupled; black dots) obtained with 150-msec trains (30 pulses). Encircled dots illustrate data obtained in entrainment paradigm (see panel B). In panel A, results are expressed as phase shifts ($\Delta\phi$'s) vs. phase (ϕ) in terms of percent of τ_{FR} . Panel B, entrainment curve obtained with 150-msec vagal trains at periods that ranged between 200 and 1200 msec. T : entrained pacemaker period expressed as percent change in free-running period (300 msec). See text for further details.

ated the pacemaker period to 1500 msec. In panel B₁, the vagal stimulus was applied at a much later phase (1140 msec); the subsequent discharge was greatly delayed. The records in panels A₂ and B₂ were obtained just before attempting entrainment, while the pacemaker beat spontaneously at a free-running period of about 1630 msec. In panel A₃, repetitive vagal stimuli were applied at a constant period of 1500 msec. No significant desensitization was apparent. When steady state was reached, the pacemaker was entrained in a 1:1 manner with each vagal stimulus occurring very early in the cycle, thus forcing the pacemaker to beat a constant period of 1500 msec. In B₃, the vagal cycle length was increased to 2200 msec; one-to-one entrainment persisted at this period. Each vagal input occurred at a much later phase (1050 msec) and, as predicted by the records in B₁, delayed the subsequent discharge by about 670 msec, entraining the pacemaker at a relatively stable cycle of 2200 msec. As in previous experiments, the characteristics of entrainment could be predicted by the PRC. The more symmetrical relation in this experiment resulted in a stable 1:1 entrainment zone that extended from

periods that were briefer to periods that were longer than the intrinsic pacemaker cycle length.

Discussion

Cardiac pacemakers can be entrained by external periodicities in their environment (Segers, 1946; Levy et al., 1969; Jalife and Moe, 1979b; Jalife et al., 1980a). In the steady state, the period of the entrained pacemaker can become identical to that of the entraining periodicity. Under these conditions, the entrained pacemaker assumes a fixed phase relationship to the entraining perturbation. Thus, entrainment of a cardiac pacemaker by an external input depends on phase control. Accordingly, one of the most important prerequisites for entrainment at various external periods is that the sensitivity of the pacemaker to the effects of the input be a function of the timing of that input.

The results of this study demonstrate conclusively that the condition of phase-dependent sensitivity is fulfilled when cardiac pacemakers interact with single or periodically applied vagal inputs. When applied periodically, brief vagal discharges are capable of

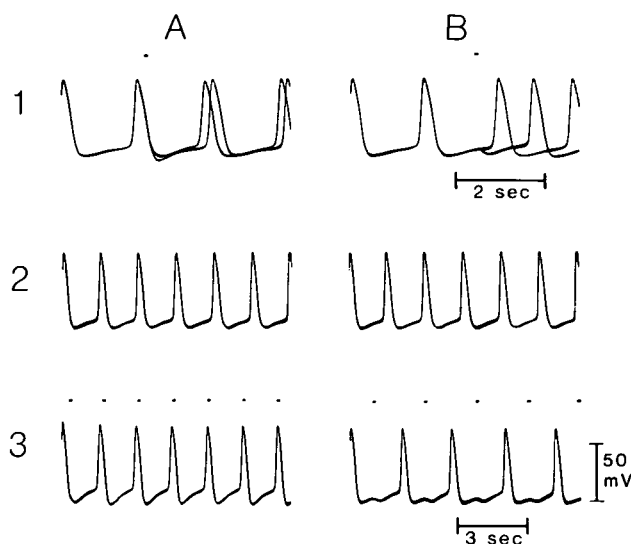


FIGURE 10. Phasic changes and entrainment of an isolated sheep sinus node preparation (mean τ_{FR} : 1630 msec). In panels A₁ and B₁, two superimposed traces are shown during control and during the application of a single (50-msec) postganglionic train at early and late phases, respectively. In panels A₂ and B₂ (note different horizontal calibration in panels A₁ and B₁), free-running periods are shown just prior to application of repetitive vagal trains at cycle lengths of 1500 and 2200 msec (panels A₃ and B₃), respectively. Stable 1:1 entrainment occurred at both vagal periods. A paradoxical acceleration is shown in panel A₃. The pacemaker was forced to beat at constant cycles that were about 130 msec briefer than τ_{FR} . In panel B₃, the same input entrained the pacemaker at periods that were 570 msec longer than τ_{FR} .

entraining the sinoatrial pacemaker because they can "correct" the pacemaker period by an amount that depends on their instantaneous relation to that period. This ability of repetitive vagal discharges to synchronize the sinoatrial pacemaker period was demonstrated in anesthetized animal preparations (Suga and Oshima, 1969; Reid, 1969; Levy et al., 1969) and was predicted by previous experiments from our laboratory in which single vagal bursts were used to scan the spontaneous pacemaker cycle in isolated vagus nerve-sinus node preparations (Jalife and Moe, 1979a).

These experiments provide definite confirmation for those predictions, and can be used to explain the cellular mechanisms involved in the phasic effects of brief vagal discharges on pacemaker periodicity. For example, as Figure 2 shows, the primary inhibitory effect of a brief vagal volley (Donders 1868; Brown and Eccles, 1934) is the result of a transient hyperpolarization that effectively delays the sinoatrial pacemaker discharge to a point that depends on its position in the spontaneous pacemaker period. This hyperpolarization is short-lived, and usually disappears during the second cycle after the stimulus (Fig. 3). In addition, as illustrated in Figures 2 and 3, under the conditions of supramaximal vagal stimulation, the absolute level of membrane hyperpolarization, as well as the interval between the stimulus and the overt

pacemaker discharge, remains relatively constant at every stimulus phase. Consequently, after strong vagal stimulation, the phase shift of pacemaker discharge induced by a brief vagal volley can be a linear function of the stimulus timing. The linearity of the relationship is dependent upon the magnitude and duration of the vagally mediated hyperpolarization.

These data provide clear and specific answers to some of the questions put forward by other authors (see Levy et al., 1969; Reid, 1969) when discussing the possible electrophysiological basis of the frequency-dependent patterns of interaction between the cardiac pacemaker and the vagus nerve. Some of those questions pertain to the cellular changes mediating the triphasic inhibitory curve described many years ago by Brown and Eccles (1934). Until recently, most of the complex series of events generated at the sinoatrial pacemaker cell membrane by the application of a brief vagal volley had not been explained satisfactorily. The evidence presented in this and other studies from our laboratory (Jalife and Moe, 1979a) indicate that the two inhibitory components on the triphasic curve are explained by two distinct mechanisms. The phase of primary inhibition is mediated by a direct hyperpolarization of the sinoatrial cell membrane that lasts about 1 second (Fig. 2) and can be approximated by comparable levels of hyperpolarization induced by current injection (Jalife and Moe, 1979a; Jalife et al., 1980a). On the other hand, the secondary inhibitory effect that appears in some rabbit and most cat SA node preparations is not associated with hyperpolarization but with a gradual decrease in the slope of phase 4 depolarization (see Jalife and Moe, 1979a) during subsequent cycles.

The primary inhibitory effect is undoubtedly triggered by acetylcholine (ACh) activation of muscarinic receptors in the external surface of the sinoatrial cell membrane (Hutter and Trautwein, 1956), and generated by a relatively fast opening of specific channels by the ACh-receptor complex (Osterrieder et al., 1980). The secondary component is probably also triggered by the ACh-receptor interaction, but it is perhaps related to intracellular metabolic processes with relatively slower kinetics, or possibly is generated by accumulation of extracellular potassium secondary to a massive permeability change (Spear et al., 1979). However, the evidence for this latter mechanism is not really convincing, since a direct correlation between secondary inhibition and external potassium activity has not been established.

One alternative explanation remains totally unexplored, namely, that the triphasic shape of the inhibitory curve may result from the interaction of ACh with more than one type (possibly two) of muscarinic receptor on the surface of the pacemaker cell membrane. This hypothesis is supported by recent biochemical experiments (Hulme et al., 1981; Galper and Smith, 1978) showing that heart pacemaker cells may possess two populations of muscarinic receptors with different binding kinetics (one fast, one slow). Additional support can be found in the original experiments of Brown and Eccles (1934), who demonstrated

that administration of small doses of atropine (5 μ g) selectively diminished the amplitude of the primary component without affecting the secondary inhibitory effect (see Brown and Eccles, 1934; their Fig. 16), therefore suggesting the possibility of two different types of receptors with different sensitivities to small doses of muscarinic antagonists.

In summary, our results provide insight into the cellular mechanism of the triphasic inhibitory curve of vagal stimulation on sinoatrial pacemaker activity. However, there exists sufficient doubt about the subcellular basis of the secondary phase of slowing to warrant further investigation.

One additional puzzle has been solved by our experiments in the isolated sinus node preparation. Ever since the classical experiments of Brown and Eccles (1934), it has been known that—under certain conditions—application of a brief vagal stimulus can unexpectedly induce acceleration of the sinoatrial pacemaker period. Until recently, no adequate explanation had been provided for this most interesting effect. Acceleration could not be the result of vagally induced pacemaker shifts; neither could it be associated with sympathetic activation, since tetanic stimulation of sympathetic nerves had been shown to abolish (Brown and Eccles, 1934)—and administration of propranolol to accentuate (Levy et al., 1969)—the acceleratory component in the triphasic curve. Our experiments provide compelling evidence in support of the hypothesis that—whenever an appropriate relationship exists between the duration of the vagally induced hyperpolarization, its timing, and the duration of the free-running pacemaker period—“paradoxical” acceleration of that period can be induced by an intrinsically inhibitory (i.e., vagal) stimulus (see Figs. 4, 5, and 10). The ionic basis of this paradoxical effect probably is related to the fact that, in cardiac tissues, hyperpolarization toward the potassium equilibrium potential decreases membrane conductance to potassium, and unmasks inward currents responsible for depolarization (c.f. Irisawa, 1978), hence leading to a postinhibitory rebound that effectively increased the slope of phase 4 depolarization. It follows that if the free-running pacemaker period is sufficiently long and if the vagal input is applied sufficiently early in the pacemaker period, the timing of the subsequent pacemaker discharge will be advanced and the pacemaker period will be abbreviated.

Under normal conditions, reflex vagal discharges are grouped in discrete bursts that occur at specific phases after every pacemaker discharge (Green, 1959; Katona et al., 1970). It is therefore obvious that the steady state PRC should be a more accurate predictor of the entrainment behavior of the cardiac pacemaker than the single train PRC. This was found to be the case in our experiments in which entrainment by postganglionic vagal stimulation was studied. Yet, our experiments show that the single train PRC can sometimes be used to anticipate the existence of entrainment, particularly at low frequencies of vagal stimulation (see Fig. 10).

These data are in complete agreement with those

of Levy and coworkers (1969, 1970a), who demonstrated that the total range of pacemaker periods covered by the steady state PRC corresponds directly with the primary range of entrainment (Figs. 8 and 9). For example, the steady state PRC of Figure 8A indicates that at no entrainment ratio in Figure 8B can the periodic vagal stimulus force the pacemaker to beat at cycles longer than 500 msec. Hence, brief vagal stimuli applied repetitively with a period somewhat longer than 500 msec could not produce stable entrainment of the pacemaker because the average ratio would fall within the zone of instability at which the vagal input would occur at various phases within the pacemaker period. Indeed, the range of stable entrainment at 1:1 is the projection of the PRC onto the pacemaker period axis (Enright, 1965).

The possible physiological and clinical implications of the entrainment phenomena demonstrated in this study have been discussed previously (Jalife and Moe, 1979a; Levy et al., 1969, 1970). Several electrophysiological studies have demonstrated that the occurrence of impulse grouping in efferent cardiac vagal fibers results in part from periodic activation by afferent baroreceptor impulses (Jewett, 1964; Kunze, 1972). Therefore, it seems likely that entrainment and vagus-SA node phase-locking play a significant role in the dynamic regulation of cardiac pacemaker activity by the autonomic nervous system. It is also possible that many sinoatrial rhythm disturbances, whether or not associated with apparent atrioventricular conduction alterations, may be the result of these frequency-dependent vagus-SA node interactions (Jalife et al., 1982; Jalife and Moe, in press). The principles may apply also to the cyclic changes of cardiac pacemaker activity induced by the respiratory rhythm. Indeed, respiratory modulation of vagal activity has been demonstrated repeatedly, and the mechanism of sinus arrhythmia has been related in one way or another to vagal activity associated with the respiratory cycle (c.f. Levy et al., 1966). As shown in Figures 7–9, in addition to the primary range of synchronization (i.e., 1:1), a sinoatrial pacemaker may have other secondary ranges of entrainment in which the pacemaker may adopt a period that is either an exact submultiple of the period of the vagal input (i.e., 2:1, 3:1, etc.) or a simple multiple of that period (i.e., 1:2, 1:3, etc.). Thus, it is quite possible that, during sinus arrhythmia, oscillatory changes in pacemaker periodicity induced by the respiratory rhythm may be associated with periodic discharge of afferent fibers in the lungs, producing brief vagal perturbations on cardiac pacemaker periodicity at relatively slow frequency. The existence of entrainment by such slow-frequency entraining cycles is in fact predicted by the PRC.

Finally, as suggested by Levy et al. (Levy and Zieske, 1970; Levy and Edlstein, 1970) one additional role for vagally mediated entrainment and phase-locking of pacemaker periodicity can be found in the distinct harmonic relations between sinoatrial and ventricular frequencies in some patients with complete AV dissociation (isorhythmic dissociation).

We wish to thank Gordon K. Moe and Irwin M. Weiner for constructive comments and for reading the manuscript. We also appreciate the technical assistance of Diana Warburton and Judith Hefferon. The help of Jimmy Megna in the statistical analysis of the data is also appreciated.

Supported by Grants HL29439 and HL06367 from the National Institutes of Health, and by Grant-in-Aid 81.1114 from the American Heart Association, with funds provided in part by the Upstate and Broome County New York Chapters.

V.A.J. Slenter was a Fellow of the Dutch Heart Foundation and SWOL Foundation. His present address is: Department of Physiology, State University of Maastricht, The Netherlands.

J. Jalife is an Established Investigator of the American Heart Association.

Address for reprints: Dr. Jose Jalife, Department of Pharmacology, S.U.N.Y. Upstate Medical Center, 766 Irving Avenue, Syracuse, New York 13210.

Received November 24, 1982; accepted for publication April 7, 1983.

References

- Aschoff J (1965) Circadian Clocks. North Holland, Amsterdam
- Bleeker WK, Mackaay AJC, Masson-Pevet M, Bouman LN, Becker AE (1980) Functional and morphological organization of the rabbit sinus node. *Circ Res* 46: 11-22
- Brown GL, Eccles JC (1934) The action of a single vagal volley on the rhythm of the heart beat. *J Physiol (Lond)* 82: 211-240
- Brown H, DiFrancesco D, Noble S (1979) Cardiac pacemaker oscillation and its modulation by autonomic transmitters. *J Exp Biol* 81: 175-204
- Donders FC (1868) Zur Physiologie des Nervus Vagus, *Pfluegers Arch Eur J Physiol* 1: 334-361
- Eckberg DL (1976) Temporal response patterns of the human sinus node to brief carotid baroreceptor stimuli, *J Physiol (Lond)* 258: 769-782
- Enright JT (1965) Synchronization and ranges of entrainment. In *Circadian Clocks*, edited by J Aschoff. Amsterdam, North Holland, pp 112-124
- Galper FB, Smith TW (1978) Properties of muscarinic acetylcholine receptors in heart cell cultures. *Proc Natl Acad Sci USA* 75: 5831-5835
- Green JH (1959) Cardiac vagal efferent activity in the cat. *J Physiol (Lond)* 149: 47P-49P
- Hill-Smith I, Purves RD (1978) Synaptic delay in the heart: An iontophoretic study. *J Physiol (Lond)* 279: 31-54
- Hulme EC, Berrie CP, Birdsall NJM, Burgen ASV (1981) Two populations of binding sites for muscarinic antagonists in the rat heart. *Eur J Pharmacol* 73: 137-142
- Hutter OF, Trautwein W (1956) Vagal and sympathetic effects on the pacemaker fibers in the sinus venosus of the heart. *J Gen Physiol* 39: 715-733
- Irisawa H (1978) Comparative physiology of the cardiac pacemaker mechanism. *Physiol Rev* 58: 461-498
- Iriuchijima J, Kumada M (1963) Efferent cardiac vagal discharge in response to electrical stimulation of sensory nerves. *Jpn J Physiol* 13: 599-605
- Jalife J, Moe GK (1979a) Phasic effects of vagal stimulation on pacemaker activity of the isolated sinus node of the young cat. *Circ Res* 45: 595-607
- Jalife J, Moe GK (1979b) A biologic model of parasystole. *Am J Cardiol* 43: 761-772
- Jalife J, Moe GK (in press) Phasic responses of SA and AV nodes to vagal stimulation. In *The Frontiers of Electrophysiology*, edited by M Rosenbaum, MV Elizari. The Hague, Martinus Nihoff
- Jalife J, Hamilton AJ, Lamanna VR, Moe GK (1980a) Effects of current flow on pacemaker activity of the isolated kitten SA node. *Am J Physiol* 238: H307-H316
- Jalife J, Hamilton AJ, Moe GK (1980b) Desensitization of the cholinergic receptor at the sinoatrial cell of the kitten. *Am J Physiol* 238: H439-H448
- Jalife J, Fraccola P, Moe GK (1982) Entrainment of the SA nodal pacemaker by brief vagal bursts in relation to AV conduction. In *Cardiac Rate and Rhythm*, edited by LN Bouman, HJ Jongasma, Martinus Nihoff. The Hague, pp 577-603
- Jewett DL (1964) Activity of single efferent fibres in the cervical vagus nerve of the dog, with special reference to possible cardioinhibitory fibres. *J Physiol (Lond)* 175: 321-357
- Katona PG, Poitras JW, Barnett GO, Terry BS (1970) Cardiac vagal efferent activity and heart period in the carotid sinus reflex. *Am J Physiol* 218: 1030-1037
- Kilbinger H, Loffelholz D (1976) The isolated perfused chicken heart as a tool for studying acetylcholine output in the absence of cholinesterase inhibition. *J Neural Transm* 38: 9-14
- Kunze DL (1972) Reflex discharge patterns of cardiac vagal efferent fibres. *J Physiol (Lond)* 222: 1-15
- Levy MN, Edlstein J (1970) The mechanism of synchronization in isorhythmic A-V dissociation. II. Clinical studies. *Circulation* 42: 689-699
- Levy MN, Zieske H (1970) Mechanism of synchronization in isorhythmic dissociation. I. Experiments on dogs. *Circ Res* 26: 429-443
- Levy MN, DeGeest H, Zieske H (1966) Effects of respiratory center activity on the heart. *Circ Res* 18: 67-78
- Levy MN, Martin PJ, Iano T, Zieske H (1969) Paradoxical effect of vagus nerve stimulation on heart rate in dogs. *Circ Res* 25: 303-314
- Levy MN, Martin PF, Iano T (1970) Effects of single vagal stimuli on heart rate and atrioventricular conduction. *Am J Physiol* 218: 1256-1262
- Martin P, Levy MN, Matsuda Y (1982) Fade of cardiac responses during tonic vagal stimulation. *Am J Physiol* 243: H219-H225
- Noma A, Irisawa H (1974) Electrogenic sodium pump in rabbit sinoatrial node cell. *Pfluegers Arch* 351: 177-182
- Osterrieder W, Noma A, Trautwein W (1980) On the kinetics of the potassium channel activated by acetylcholine in the SA node of the rabbit heart. *Pfluegers Arch* 386: 101-109
- Perkel DH, Schulman JH, Bullock TH, Moore GP, Segundo JP (1964) Pacemaker neurons: effect of regularly spaced synaptic input. *Science* 145: 61-63
- Pinsker HM (1977) Synaptic modulation of endogenous neuronal oscillators. *Fed Proc* 36: 2045-2049
- Pittendrigh CA (1965) On the mechanism of entrainment of a circadian rhythm by light cycles. In *Circadian Clocks*, edited by J Aschoff. Amsterdam, North Holland, pp 277-297
- Reid JVO (1969) The cardiac pacemaker: Effects of regularly spaced nervous input. *Am Heart J* 78: 58-64
- Satow Y (1968) Hyperpolarization of the quiescent heart and inhibition of the heart beat by vagal impulses. *Tohoku J Exp Med* 94: 241-256
- Segers M (1946) Les phenomenes de synchronisation au niveau du coeur. *Arch Int Physiol* 54: 87-105
- Spear JF, Kronhaus KD, Moore EN, Kline RP (1979) The effect of brief vagal stimulation on the isolated rabbit sinus node. *Circ Res* 44: 75-88
- Steel RGD, Torrie JH (1980) Principles and Procedure of Statistics. New York, McGraw-Hill
- Suga H, Oshima M (1968) Modulation characteristics of heart rate by vagal stimulation. *Jpn J Med Electron Biol* 6: 465-471
- Suga H, Oshima M (1969) Periodic variation of heart rate caused by repetitive electric stimulation of cardiac vagus nerve. *J Physiol Soc Jpn* 31: 33-34
- Tokimasa T, Hassuo H, Koketsu K (1980) Desensitization of the muscarinic receptor of bullfrog atrial muscle. *Experientia* 36: 1200-1201
- Vincenzi FF, West TC (1963) Release of autonomic mediators in cardiac tissue by direct subthreshold electrical stimulation. *J Pharmacol Exp Ther* 141: 185-194
- Wallenstein S, Zucker CL, Fleiss JL (1980) Some statistical methods useful in circulation research. *Circ Res* 47: 1-9
- Yanagihara K, Noma A, Irisawa H (1980) Reconstruction of sinoatrial node pacemaker potential based on the voltage clamp experiments. *Jpn J Physiol* 30: 841-857

INDEX TERMS: Heart rate · Entrainment · Synchronization · Acetylcholine · Parasympathetic reflexes