Effects of Sympathetic Tone on Vagally Induced Phasic Changes in Heart Rate and Atrioventricular Node Conduction in the Anesthetized Dog

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SUMMARY. We examined the effects of stellate ganglia stimulation on the phase-dependent chronotropic and dromotropic responses to brief vagal bursts in open-chest anesthetized dogs. Stellate stimulation affected the phasic vagal effects on heart rate by shortening the latent period, shifting the phase at which maximum decrease in heart rate occurred to earlier phases, and reducing the maximum decrease in heart rate. These effects were due primarily to an increase in the basic heart rate. No significant sympathetic-parasympathetic interaction occurred for heart rate, indicating that accentuated antagonism did not occur with brief vagal bursts. Stellate stimulation primarily decreased the amplitude of the phasic vagal effects on atrioventricular nodal conduction, regardless of the underlying heart rate, and a significant sympathetic-parasympathetic interaction was associated with this effect. The peak of the phase-dependent vagal effects on heart rate and atrioventricular nodal conduction were phase-shifted with one another. From these findings, we postulate the small changes in sympathetic tone might shift the predominant phase-dependent vagal effect from one on heart rate to one on atrioventricular nodal conduction. Furthermore, our results suggest that dynamic vagal control of heart rate and atrioventricular node conduction involves both phase-dependent and phase-independent factors. Sympathetic activity appears to affect only the phase-independent factor(s) in the control of heart rate, whereas it affects both phase-dependent and phase-independent factors in the control of atrioventricular node conduction. (Circ Res 58: 584-594, 1986)

BOTH the sympathetic and parasympathetic branches of the autonomic nervous system exert a profound influence on sinoatrial (SA) and atrioventricular (AV) node function, thereby regulating heart rate and AV node conduction. Activation of sympathetic nerve fibers may exaggerate or blunt cardiovascular responses to parasympathetic activity. For example, tonic vagal stimulation produces a greater absolute reduction in heart rate in the presence of tonic background stellate stimulation (Rosenblueth and Simeone, 1934; Warner and Russell, 1969; Levy and Zeiske, 1969), a sympathetic-parasympathetic interaction that has been termed "accentuated antagonism" (Levy, 1971). In contrast, changes in AV conduction during concomitant sympathetic and parasympathetic stimulation are essentially the "algebraic sum" of the individual AV conduction responses to tonic vagal and stellate stimulation alone (Levy and Zeiske, 1969; Wallick et al., 1982).

Cardiac responses to brief vagal bursts begin after a short latency and dissipate quickly. In comparison, the cardiac responses to sympathetic stimulation arise very slowly and dissipate over relatively long periods of time, such that the primary vagal effect may run its full course during the latency of the response to stellate stimulation. The rapid onset and offset of responses to vagal stimulation allow for dynamic vagal modulation of heart rate and AV conduction, whereas the slow temporal response to stellate stimulation precludes any beat-to-beat regulation by sympathetic activity (Warner and Russell, 1969; Spear and Moore, 1973). As a result, the chronotropic (Brown and Eccles, 1934; Levy et al., 1970; Jalife and Moe, 1979) and dromotropic (Martin, 1977) responses to brief vagal stimuli are markedly phase-dependent, while the responses to stellate stimulation are phase-independent (Levy and Zeiske, 1969; Spear and Moore, 1973).

Although the dynamic interactions between brief vagal activity and control of heart rate and AV conduction have been studied in some detail, there is little information on the effect of coincident sympathetic activity on these phase-dependent interactions. In a recent study, Stuesse et al. (1981) examined the effects of tonic stellate stimulation on the chronotropic responses to repetitive phase-coupled vagal bursts. They demonstrated that small changes in sympathetic tone may greatly alter the chronotropic sinus node response to phasic vagal stimulation, especially if the vagal bursts appear at critical times within the cardiac cycle (Yang and Levy, 1984). However, the effect of stellate stimulation on dromotropic AV node responses were not investi-
gated. The purpose of the present study was to examine the effect of coincident sympathetic tone on the phase-dependent chronotropic and dromotropic responses to brief vagal stimulation in open-chest anesthetized dogs.

**Methods**

**Surgical Procedures**

Mongrel dogs of either sex that weighed between 18 and 26 kg were anesthetized with thiopental sodium (15 mg/kg, iv) and α-chloralose (75 mg/kg, iv). Supplemental doses of α-chloralose were given as needed to maintain anesthesia during the experiments. No data were obtained for at least 15 minutes after each additional dose of α-chloralose, and intervening dosages were avoided during the serial measurements. Positive pressure ventilation was applied through auffed endotracheal tube with a Harvard volume-cycled respirator. Positive end expiratory pressure was used to maintain PEEP greater than 80 torr, and dogs were given sodium bicarbonate as needed to maintain the pH above 7.3. Pco₂ was kept above 30 torr by adjusting the tidal volume of the respirator. Body temperature was monitored and maintained at 37.5°C with a thermostatically controlled heating blanket placed beneath the dog. The left femoral vein was cannulated, and Ringer’s lactate solution was infused at a rate of 100 ml/hr to replace spontaneous fluid losses. A heparinized saline-filled cannula was placed in the left femoral artery and connected to a pressure transducer (Statham P-23) to monitor arterial blood pressure. A mid-sternal thoracotomy was performed, the pericardium was opened, and the cut edges were sutured to the wound edges to support the heart. The open chest was covered with a plastic sheet, and epicardial temperature was monitored and maintained at 37°C by adjusting the distance of an operating table lamp from the chest.

**Stellate and Vagal Stimulation**

The afferent connections of both stellate ganglia were isolated, doubly ligated, and cut. Shielded bipolar platinum electrodes were looped underneath both ansae subclaviae and connected to separate constant current stimulus isolators (model 850) driven by a programmable interval generator (model 830, W-P Instruments). Stimuli were rectangular pulses 4 msec in duration, 2 mA in amplitude. The intensity of stellate stimulation was varied by changing the frequency of stimulation. Both cervical vagi were isolated, doubly ligated, and cut to remove tonic vagal influences. Bipolar intradine wires, insulated except for the tip, were inserted inside the sheath of the nerve with a 23-gauge needle. Stimulating electrodes were connected to separate constant current sources driven by a Frederick Haer Pin stimulator. Stimuli were 2-msec rectangular pulses with a current strength equal to 75% of that which produced complete asystole or AV block at lead II surface electrocardiogram, arterial blood pressure, and vagal and stellate stimulus markers were recorded on an eight-channel oscillograph (Honeywell 1858 CRT Vis- corder). Measurements were made from the recordings with a Hewlett-Packard computer (model 9826) and digitizer pad (model 9874A), and were reproducible with an error of less than 2 msec. The free-running or basic cardiac cycle length (BCL) was defined as the interval between two successive atrial (A) waves, beginning at the earliest deflection in the high right atrial electrogram. Thus, in these studies, we monitored sinus rate, hereafter generally termed heart rate or inversely cardiac cycle length (A-A interval). The vagal stimulus position or phase (Φ) was defined as the interval between the previous A-wave and the beginning of the vagal stimulus. Steady state cardiac cycle lengths were established either in the absence or presence of background tonic stellate stimulation (1 or 2 Hz). Then, the cardiac cycle was scanned with brief bursts of vagal stimuli (1 or 2 pulses) that were applied either singly every 60 seconds or were coupled, repetitively, at varying intervals to each previous A-wave (phase-coupling; Jalife et al., 1983).

**AV Node Conduction Studies**

A 6 French bipolar catheter (electrodes 10 mm apart) was passed via the right carotid artery to the noncoronary cusp of the aorta to record activity from the His bundle. Sinus nodal automaticity was suppressed by infusion of verapamil (0.5–2.0 mg) into the sinus node artery (Zipes and Fischer, 1974). This procedure suppressed the sinoatrial (SA) node pacemaker without affecting AV node conduction or the vagal and sympathetic nerves traversing the SA node region. The right atrium was paced at constant cycle lengths of 300–600 msec using a bipolar plunge electrode inserted into the sulcus terminalis. Pacing stimuli were 2-msec rectangular pulses with a current intensity of 1.5 times threshold. Local low right atrial bipolar, right ventricular bipolar, and His bundle electrograms and lead II surface electrocardiograms, arterial blood pressure, and stellate and vagal stimulus markers were recorded and measured as in the sinus rate studies. The atrio-His (A-H) interval was measured as the time between the earliest atrial and His deflections in the His bundle electrogram. Steady state AV node conduction times (A-H intervals) were established either in the absence or presence of background tonic stellate stimulation (1–4 Hz). The cardiac cycle was scanned with vagal stimuli, as described above, using the same protocol as in the sinus rate studies.

Using the standard vagal stimulation protocol as described above, AV block occurred in half (3/6) of the experiments during application of single vagal stimuli (cf. Fig. 4A). As a result, quantitative statistical comparison of the data for single vs. repetitive phase-coupled vagal stimuli was not possible. Therefore, in an additional series of experiments, the vagal stimulus current intensity was adjusted so that optimum prolongation of the A-H interval was obtained without inducing AV block with either mode of vagal stimulation at the higher stimulus level (2 pulses). In addition, the pacing cycle length (PCL) was varied over the range of 300–600 msec in 50- or 100-msec steps. Complete sets of data (i.e., all combinations of independent variables) were obtained for as many pacing cycle lengths as possible (usually 400 and 500 msec) in any given experiment.

**Phase-Response Curve Parameters**

A phase-response curve (PRC) graphically depicts the effects of brief vagal perturbations on cardiac cycle length
or AV node conduction time. Figure 1 shows typical PRC for cardiac cycle length. The A-A interval is plotted on the ordinate as a function of the position or phase (\( \Phi \)) of the vagal stimulus in the cardiac cycle. The A-A interval prolongs as a function of the vagal stimulus phase until it reaches a maximum, then rather abruptly drops to a minimum. These maximum and minimum points were chosen as critical values for quantitative analysis of the effect of varying intensities of stellate and vagal stimulation as well as the mode of vagal stimulation on the vagally induced PRC. Thus, \( \Phi_{\text{max}} \) and \( \Phi_{\text{min}} \) (cf. Fig. 1A, filled circles) correspond to the vagal phases at which the respective maximum (\( A_{\text{max}} \)) and the minimum (\( A_{\text{min}} \)) effects on the A-A interval occur. The difference between \( \Phi_{\text{max}} \) and \( \Phi_{\text{min}} \) defines \( \Phi_{\text{dif}} \) or the duration of the negative slope region of the PRC, and the difference between \( A_{\text{max}} \) and \( A_{\text{min}} \) defines the PRC amplitude. Analogous points of quantitation apply to the AV conduction PRC.

"Single PRC" (Levy et al., 1970; Jalife et al., 1983) are plots of the effect of a single vagal stimulus on the immediate A-A interval only (Fig. 1A). "Phase-coupled PRC" (Jalife et al., 1983) are plots of the steady state A-A interval during vagal stimulation repeated in each cardiac cycle (Fig. 1B). The latent period is the interval between the earliest single vagal stimulus that fails to prolong the immediate cardiac cycle and the end of that cycle. By definition, then, the latent period exists only for single PRC and corresponds to the length of the later flat portion of the single PRC (Fig. 1A).

In the AV node conduction studies, single PRC are plots of the A-H interval of the first poststimulus cycle, since the control A-H intervals are less than the latent period for the vagal effect (Martin, 1975). Phase-coupled PRC are plots of the steady state A-H interval during vagal stimulation repeated in each cardiac cycle.

### Statistical Methods

Data are expressed as mean ± SEM. Linear regression was used to examine the relationship between two continuous variables. Three-way analysis of variance (ANOVA) was used to analyze simultaneously the effects of stellate stimulation, vagal stimulation, and single vs. repetitive phase-coupled vagal stimulation on the parameters of the PRC. For each of these conditions, a significant \( P \) value indicates that the condition affects the value of the dependent variable. In addition, analysis of covariance (ANCOVA) was done using the three factors above, with cycle length as a covariate. Loss of significance of a factor with the addition of the covariate indicates that the factor effect can be explained by cycle length changes (Snedecor and Cochran, 1967).

### Results

**Effects on Cardiac Cycle Length**

Figure 1 shows examples of the effects of tonic stellate stimulation on the phasic cardiac cycle length responses to brief single and repetitive vagal stimulus bursts of 2 pulses each. Typically, in the single PRC (Fig. 1A), as the vagal stimulus phase was progressively changed, the A-A interval increased to a maximum (\( \Phi_{\text{max}} \)), then abruptly became unaffected (\( \Phi_{\text{min}} \)), and the phase-dependent vagal effect was postponed to the following cardiac cycle. As is evident from the ordinate value during the late flat portion of the plot (latent period), the free-running cardiac cycle length was decreased from 570 to 465 and 400 msec by tonic stellate stimulation at frequencies of 1 and 2 Hz, respectively. Phase-coupled PRC (Fig. 1B) differed from single PRC. The slope of the rising phase usually was less, and the transition between the maximum and minimum points was gradual, as opposed to abrupt; thus \( \Phi_{\text{dif}} \) was greater for phase-coupled than for single PRC. \( \Phi_{\text{max}} \) occurred at slightly later phases, as indicated by the arrows pointing to the comparable \( \Phi_{\text{max}} \) value for the single PRC, whereas \( \Phi_{\text{min}} \) occurred at much later phases. \( A_{\text{max}} \) and \( A_{\text{min}} \) values were greater for phase-coupled than for single PRC, but the amplitude of each phase-coupled PRC was reduced, as compared to the respective single PRC. These results are similar to those observed previously in vitro in isolated sinoatrial node preparations (Jalife et al.,
Stellate stimulation shifted the PRC to earlier phases and shorter cycle lengths, and decreased the PRC amplitude in a frequency-dependent manner.

The mean data from nine similar experiments are depicted in Figures 2 and 3, and the statistical analysis of the data is given in Table 1 (ANOVA). In each panel of Figures 2 and 3, the effects of three independent parameters were compared for their influence on one of the dependent parameters of the PRC defined previously. The effect of three levels of stellate stimulation (0, 1, and 2 Hz) was examined, and at each of these levels the effect of vagal stimulation at two different intensities (bursts of 1 or 2 pulses) was determined. Finally, the two modes of vagal stimulation, namely, single vs. repetitive phase-coupled stimulation, were compared. Stellate stimulation significantly decreased $\Phi_{\text{max}}$, $\Phi_{\text{min}}$, $A_{\text{max}}$, and $A_{\text{min}}$ in a frequency-dependent manner, and the reduction tended to be greater for single than for phase-coupled PRC. Although the amplitude of phase-coupled PRC tended to decrease with stellate stimulation (Fig. 3), overall there was no significant effect of stellate stimulation on PRC amplitude. In contrast, all the above PRC parameters were increased significantly by increasing the intensity of vagal stimulation. All the PRC parameters were greater for phase-coupled than for single PRC, except for PRC amplitude, which was significantly less for phase-coupled than for single PRC. ANOVA indicated that all interactions between the independent variables were not significant.

The data in Figures 1 and 2 suggested that the magnitudes of $A_{\text{max}}$ and $A_{\text{min}}$ depended on the absolute phase at which these values occurred. Regression analysis of phase-coupled PRC data revealed a linear correlation between $\Phi_{\text{max}}$ and $A_{\text{max}}$ ($A_{\text{max}} = 1.94 \Phi_{\text{max}} + 306, r = 0.81; P < 0.001$), and also between $\Phi_{\text{min}}$ and $A_{\text{min}}$ ($A_{\text{min}} = 1.35 \Phi_{\text{min}} + 71, r = 0.92; P < 0.001$).
Effects on the Latent Period

Stellate stimulation significantly ($P < 0.005$) shortened the latent period in a frequency-dependent manner (not shown). The latent period (mean ± SEM) equaled 170 ± 8, 149 ± 5, and 138 ± 5 msec at stellate stimulation levels of 0, 1, and 2 Hz, respectively. Regression analysis demonstrated a significant linear correlation ($r = 0.75; P < 0.001$) between the latent period and the basic cycle length over the range of 230–550 msec. The effect of stellate stimulation on the latent period was nonsignificant ($P > 0.1$) when analyzed with basic cycle length as the covariate (Table 1, ANCOVA).

Effects on AV Conduction

Representative examples of the effects of tonic stellate stimulation on AV node conduction PRC are shown in Figure 4. Data were obtained during atrial pacing at a constant cycle length of 500 msec. During vagal stimulation alone (Fig. 4A, filled circles), AV block (indicated by the arrows) occurred in this single PRC near $\Phi_{\text{max}}$. Tonic stellate stimulation at frequencies of 1, 2, and 4 Hz reduced the control A-H interval from 92 to 82, 60 and 52 msec, respectively. Stellate stimulation produced a small but significant decrease in $\Phi_{\text{max}}$ and reduced the prolongation of the A-H interval at all vagal stimulus phases, but the degree of this antagonism was greater when the phasic vagal effect was maximal ($\text{AH}_{\text{max}}$) than when it was minimal ($\text{AH}_{\min}$), resulting in a marked decrease in PRC amplitude. Indeed, at higher frequencies (4 Hz) of stellate stimulation (unfilled squares), the single PRC was nearly flattened. Tonic stellate stimulation also reduced $\text{AH}_{\text{max}}$, $\text{AH}_{\text{max}}$, and the amplitude of the phase-coupled PRC (Fig. 4B). Quantitative substantiation of these effects is given in Figure 5.

Figure 6 shows the quantitative AV conduction data from 10 experiments in which the vagal stimulus current intensity was modified as described in Methods. The effects on four dependent PRC parameters—namely, $\Phi_{\text{max}}$, $\text{AH}_{\text{max}}$, $\text{AH}_{\min}$, and PRC amplitude—are shown, and the statistical analysis is given in Table 2 (ANOVA). Tonic stellate stimulation alone at frequencies of 1 and 2 Hz decreased the A-H interval (mean ± SEM) from 83 ± 3 to 69 ± 3 and 60 ± 3 msec, respectively. Stellate stimulation
significantly decreased all PRC parameters in a frequency-dependent manner. Raising the level of vagal stimulation significantly increased all PRC parameters, but tended to increase $\Phi_{\text{max}}$ only for phase-coupled PRC. All the parameters except PRC amplitude were significantly greater for phase-coupled than for single PRC. However, the absolute differences were much less for $A_{\text{Hmax}}$ than for $A_{\text{Hmin}}$. Indeed, $A_{\text{Hmax}}$ was sometimes smaller for the phase-coupled than for the single PRC. Furthermore, the increase in $A_{\text{Hmin}}$ produced by raising the level of vagal stimulation (2 pulses vs. 1 pulse) was significantly greater for phase-coupled than for single PRC (interaction term: VS X [S vs. PC]). As a result, and in contrast to the other parameters, PRC amplitude was always greater for the single than for phase-coupled PRC, and the difference was larger at the higher level of vagal stimulation (interaction term: VS X [S vs. PC]). There also was a significant interaction between both the level and the mode of vagal stimulation and the level of stellate stimulation. In other words, the increase in PRC amplitude induced by raising the level of vagal stimulation was greater at lower stellate stimulation levels, and the difference between the PRC amplitude for single vs. phase-coupled PRC was larger at lower levels of stellate stimulation.

**Effects of Pacing Cycle Length on AV Node Conduction PRC**

Figure 7 shows single (panel A) and phase-coupled (panel B) PRC obtained at four different pacing cycle lengths. The control A-H intervals were 53, 58, 63, and 70 msec at pacing cycle length’s of 500, 450, 400, and 350 msec, respectively. Decreasing the pacing cycle length shifted the PRC upward and to the left, regardless of the mode of vagal stimulation. Thus, as the pacing cycle length was decreased, $\Phi_{\text{max}}$ was decreased significantly (see also Fig. 8), whereas $A_{\text{Hmax}}$ and $A_{\text{Hmin}}$, and PRC amplitude were significantly increased (Table 2, ANCOVA). The previously described differences between the single and phase-coupled PRC were readily apparent at
any given pacing cycle length, as were the effects of varying the level of stellate stimulation and vagal stimulation (Table 2, ANCOVA).

### Cardiac Cycle Length vs. AV Conduction PRC

The PRC for cardiac cycle length and AV conduction were phase-shifted with one another (Fig. 8).

**Table 2**

Results of Statistical Analysis on the Parameters of the PRC for AV Node Conduction

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\( n = 8 \); abbreviations same as in Table 1.

\( \ast \) Interaction terms that were significant.

For any cycle length (CL), the \( \Phi_{max} \) value of the PRC for AV conduction was greater than the \( \Phi_{max} \) for cardiac cycle length. Thus, the peak effect of brief vagal stimulation on AV conduction and the entire PRC (Fig. 9) occurred at later phases than did the vagal effects on cardiac cycle length. The difference between the cardiac cycle length and AV conduction \( \Phi_{max} \) values was larger at longer cycle lengths (150 msec at CL = 600 msec vs. 80 msec at CL = 300 msec).

### Discussion

The results of this study indicate that single or phase-coupled vagal stimuli produced phase-dependent increases in cardiac cycle length and AV node conduction. Coincident stellate stimulation affected the phasic vagal effects on cardiac cycle length by shortening the latent period, shifting the phase at which maximum cardiac cycle length prolongation occurred to earlier phases, and reducing the maximum increase in cardiac cycle length. These effects appeared to be due almost entirely to a decrease in the basic cardiac cycle length during stellate stimulation. Stellate stimulation primarily decreased the amplitude of the phasic vagal effects on AV node conduction, regardless of the underlying cardiac cycle length. Therefore, the sympathetic effects could not be accounted for simply on the basis of a decrease in cardiac cycle length. The peak of the phase-dependent vagal effects on cardiac cycle length and AV node conduction were phase-shifted with one another. As a result, small changes in sympathetic tone might shift the predominant phase-dependent vagal effect from one on cardiac cycle length to one on AV node conduction.

Our results suggest further that dynamic vagal
control of cardiac cycle length and AV node conduction involves both phase-dependent and phase-independent factors. Sympathetic activity appears to affect only the phase-independent factor(s) in the control of cardiac cycle length, whereas it affects both phase-dependent and phase-independent factors in the control of AV node conduction. The differences between the sympathetic effects on the cardiac cycle length and AV node conduction PRC suggest that there is more than one mechanism responsible for the dynamic phase-dependent vagal control of cardiac cycle length and AV node conduction.

Effects of Sympathetic Stimulation on Cardiac Cycle Length PRC

As expected, sympathetic tone significantly affected the basic cycle length in our experiments. Since previous experiments in vitro demonstrated that the PRC parameters depended not only on the position, intensity, and duration of the vagal stimulus, but also on the prevailing basic cycle length (Jalife and Moe, 1979; Jalife et al., 1983; Michaels et al., 1983), we analyzed our data using ANCOVA to test whether the effects of sympathetic stimulation on the PRC parameters occurred as a direct consequence of the changes in the basic cycle length or as a result of other sympathetic-parasympathetic interactions. ANCOVA revealed that the statistically significant effects of stellate stimulation on the PRC parameters were either greatly reduced ($A_{max}$) or were rendered nonsignificant by inclusion of the basic cycle length as a covariate. We conclude that the sympathetic-induced changes in the PRC were due primarily to a change in basic cycle length. Yang et al. (1985) recently showed analogous cycle length-dependent changes in phase-coupled PRC. However, they varied the basic cycle length with tonic vagal stimulation alone or in combination with cooling of the SA node region. Thus, regardless of the manner by which basic cycle length is varied, it appears to be a primary determinant of the characteristics of the vagally induced phasic cycle length responses.

In these experiments using brief vagal bursts, there was no evidence for "accentuated antagonism" between sympathetic and parasympathetic effects on cardiac cycle length as previously described by Levy (1971) for tonic vagal stimulation. All interaction terms were not significant (Table 1). During stellate stimulation, the frequency of phase-coupled vagal bursts increased as a result of the decrease in cardiac cycle length and should have produced an
FIGURE 9. Postulated changes in cardiac cycle length and AV node conduction during small changes in sympathetic tone coincident with brief vagal bursting. Cardiac cycle length PRC (A-A intervals, solid lines) and the top AV node conduction PRC (A-H intervals, dashed lines) were generated experimentally using vagal bursts of 2 pulses each. The top curves were obtained in the absence of stellate stimulation at basic free-running (solid line) and pacing cycle lengths (dashed line) of approximately 600 msec. The bottom cardiac cycle length PRC resulted during stellate stimulation (+SS) at a frequency of 1 Hz. The bottom AV node conduction PRC is a hypothetical curve derived by combining the expected direct and "indirect" effects of stellate stimulation on the A-H interval. The direct effect is a decrease in PRC amplitude, while the indirect effects mediated by a decrease in cardiac cycle length are an increase in PRC amplitude and a shift to shorter cycle lengths (see previous description in the Results, and Figs. 4-7). Intervals are expressed in milliseconds. See text for further details.

increase in the vagal effect, yet PRC amplitude was reduced. One might conclude that there was a greater sympathetic antagonism under these circumstances, but fade of the vagal effect is an alternative explanation (see discussion of single vs. repetitive vagal stimulation below). Slightly higher levels of vagal (four pulses) and stellate (4 Hz) stimulation produced similar results (not shown). Although the cycle length changes we observed covered a major portion of the physiological range, we cannot exclude the possibility that sympathetic-parasympathetic interactions might have been manifest at higher levels of neural activity.

Effects of Sympathetic Stimulation on the Latent Period

In these experiments, the latent period, defined as the time between the onset of the earliest vagal stimulus which failed to prolong the immediate cardiac cycle and the end of that cycle, was decreased significantly by stellate stimulation in a frequency-dependent manner. The latent period varied as a function of the basic cycle length, and regression analysis revealed that this relationship was fit best by a straight line over the range of cycle lengths examined (230–550 msec). In the classic study by Brown and Eccles (1934), the latent period shortened when the basic cycle length was decreased by stellate stimulation and, conversely, the latent period lengthened when the basic cycle length was increased by background tetanic vagal stimulation. Spear and Moore (1973) found that the duration of the latent period increased as the hierarchy of subsidiary pacemaker activity decreased (sinus node < AV junctional < atrial ectopic < ventricular ectopic), and there was a rough correlation between latent period and basic cycle length. However, it is likely that the relative density of parasympathetic nerve terminals in the different pacemaker regions (see Brown et al., 1985) also was an important contributing factor. Alternatively, alterations in sinoatrial conduction time might explain the changes in the latent period. The mechanism(s) responsible for the cycle length-dependent changes in the latent period, and especially those induced by alterations in sympathetic tone, are undetermined.

During repetitive (phase-coupled) vagal stimulation, Stuesse et al. (1981) found that stellate stimulation produced a small but significant decrease in the time from the vagal stimulus that evoked the maximum cardiac cycle length response until the subsequent P-wave (St-P of P-P_max). St-P of P-P_min, which corresponds to the latent period, was unaffected by stellate stimulation. We observed similar results in this study (not shown). However, we found that, during phase-coupled vagal stimulation, St-P of P-P_max correlates more closely with the latent period (see Fig. 1), due to the increase in $\Phi_{dir}$. Thus, a decrease in St-P of P-P_max is indicative of a decrease in the latent period.

Effects of Sympathetic and Vagal Stimulation on AV Node Conduction PRC

These experiments are the first to describe the effects of coincident sympathetic activity on the dynamic phase-dependent vagal effects on AV node conduction. Stellate stimulation significantly decreased all the AV node conduction PRC parameters, regardless of pacing cycle length (Table 2). The most outstanding effect was a decrease in the PRC amplitude (Figs. 4–6), in contrast to the minor nonsignificant decrease in this parameter for the cardiac cycle length PRC. A significant sympathetic-parasympathetic interaction occurred which had an effect on the AV node conduction PRC amplitude; i.e., raising the level of vagal stimulation produced a greater effect at lower levels of stellate stimulation. Therefore, the interaction was not merely additive, as was found with tonic vagal stimulation (Levy and Zeiske, 1969; Wallick et al., 1982). There was no significant interaction for the other PRC parameters. The reasons for these apparent discrepancies are unclear. However, it may be that PRC amplitude is the best indicator for the phase-dependent vagal mechanism(s), while the other PRC parameters largely reflect phase-independent vagal effects.
which would predominate during tonic vagal stimulation. Thus, the significant sympathetic-parasympathetic interaction may occur only for the phase-dependent mechanism.

**Pacing Cycle Length and the AV Node Conduction PRC**

The experiments on AV node conduction were done during constant atrial pacing to eliminate the cycle length-dependent changes in AV node conduction time. When we varied the pacing cycle length, the amplitude of the vagal effects was greater at shorter pacing cycle lengths, and the PRC were shifted to longer A-H intervals and shorter cycle lengths (Fig. 7). During normal physiological conditions, an increase in sympathetic tone should shorten the basic cycle length, which would prolong the A-H interval indirectly, and augment the phasic vagal effect. At the same time, increased sympathetic activity would shorten the A-H interval directly, and ideally maintain AV node conduction time within normal limits. Thus, these complex and dynamic interactions operate normally in harmony with one another to assure proper AV node conduction time and adequate cardiac function. Certain anomalies, such as an imbalance between the autonomic branches, or an abnormal sympathetic-parasympathetic interaction, or improper timing of vagal bursts could result in abnormal AV node conduction and cardiac arrhythmias.

**Cardiac Cycle Length vs. AV Node Conduction PRC**

The PRC for cardiac cycle length and AV node conduction were phase-shifted with one another, as shown in previous studies (Jalife et al., 1982, 1984; Slentz et al., 1984). At any given underlying cycle length, the peak effect ($\Phi_{max}$, Fig. 8), and, indeed, the entire PRC for AV node conduction, occurred at later phases in the cardiac cycle than those for cardiac cycle length (Fig. 9). Assuming a constant latency for the baroreflex arc, the timing of a vagal burst would remain constant, and if the vagal effect occurred near the peak of the PRC, a change in sympathetic tone could shift the predominant vagal effect from a change primarily exerted on cardiac cycle length to a change primarily exerted on AV node conduction. For example, in Figure 9 at an "intrinsic" cardiac cycle length of about 600 msec, the upper PRC would be expected at a given vagal stimulus intensity. Vagal bursts at a phase of 350 msec predominantly would prolong the cardiac cycle length (A) which would shorten the A-H interval. An increase in sympathetic tone (+SS) would decrease cardiac cycle length, and thereby shift both the cardiac cycle length and AV node conduction PRC as demonstrated in this study. If the vagal stimulus phase is relatively unchanged, the predominant effect would be prolongation of the A-H interval, since the vagal burst now would be positioned at $\Phi_{max}$ for AV node conduction (B), but $\Phi_{min}$ for cardiac cycle length. The latter result might further augment the vagal effect on AV node conduction due to an additional decrease in cardiac cycle length. Thus, it is hypothetically possible for a small increase in sympathetic tone to produce a paradoxical increase in AV node conduction time, as a result of a small chronotropic effect. In the extreme case, if coincident vagal activity is large, a small increase in sympathetic tone could produce AV block. Conversely, a small decrease in sympathetic tone (−SS) could result in a very large negative dromotropic effect if coincident vagal bursts are initially positioned at $\Phi_{min}$ (C), since the vagal effect would shift to $\Phi_{max}$ (D) as a result of a small increase in the basic cycle length. This "accentuated antagonism" of sympathetic-parasympathetic interaction for dromotropic responses would be analogous to that recently demonstrated for chronotropic responses by Yang and Levy (1984).

**Single vs. Repetitive Phase-Coupled Vagal Stimulation**

Both cardiac cycle length and AV node conduction PRC parameters were almost all greater for phase-coupled than for single vagal stimulation. This resulted from accumulation of the longer-lasting secondary inhibitory effects after each vagal stimulus (Jalife et al., 1983), until a steady state was reached. A notable exception was PRC amplitude, which consistently was less with phase-coupled vagal stimulation. Moreover, this effect occurred for both cardiac cycle length and AV node conduction PRC. This difference occurred primarily as a result of “fade” of the vagal responses, and confirms in vivo previous findings in vitro (Jalife et al., 1980; Salata and Jalife, 1985). Furthermore, these results suggest a time-dependent interaction between (at least) two mechanisms responsible for the vagally mediated negative chronotropic and dromotropic effects. One is phase-dependent, has a rapid onset, and “fades” with time. Another is phase-independent, has a slower onset, and accumulates with time. Preliminary evidence in support of this hypothesis was presented recently (Salata et al., 1985).

We thank Dr. Naomi Fineberg for performing the statistical analyses and Becky Russell and Jan Davis for assisting in some of the experiments.

Supported in part by the Herman C. Kranert Fund, Indianapolis, Indiana; by Grants HL-06367, HL-06308, and HL-07182 from the National Heart, Lung, and Blood Institute of the National Institutes of Health, Bethesda, Maryland; by the Attorney General of Indiana Public Health Trust and by the Bouchedue Veterans Administration Medical Center, Indianapolis, Indiana; and by a Grant-in-Aid from the American Heart Association, Indiana Affiliate, Inc., Indianapolis, Indiana.

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Received July 29, 1985; accepted for publication January 9, 1986.
References


INDEX TERMS: Autonomic nervous system • Sympathetic-parasympathetic interaction • Heart rate • AV node conduction • Phase response curves
Effects of sympathetic tone on vagally induced phasic changes in heart rate and atrioventricular node conduction in the anesthetized dog.

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*Circ Res*. 1986;58:584-594
doi: 10.1161/01.RES.58.4.584

*Circulation Research* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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