Sequence of Excitation as a Factor in Sympathetic–Parasympathetic Interactions in the Heart

Tianen Yang and Matthew N. Levy

We determined the influence of differences in the time of initiation of sympathetic and vagal stimulation (both at 10 Hz) on the cardiac autonomic interactions in 16 open-chest anesthetized dogs. We always ended the concurrent sympathetic and vagal stimulations simultaneously. Sympathetic stimulation alone for 1 minute increased heart rate by 90±7 (mean±SEM) beats per minute, and vagal stimulation alone for 1 minute decreased heart rate by 67±5 beats per minute; i.e., the algebraic sum of these responses was an increase of 23 beats per minute. However, combined sympathetic and vagal stimulation for 1 minute actually decreased heart rate by 35 beats per minute; i.e., the vagal effects predominated. When vagal stimulation was initiated first, the chronotropic responses to combined stimulation were not significantly affected by the duration of antecedent vagal stimulation. However, when sympathetic stimulation was initiated first, the vagal predominance (disparity between the summated individual responses and the combined response) progressively diminished as we increased the duration of antecedent sympathetic stimulation. The vagal predominance diminished from a value of 67±21 beats per minute when the stimulations were initiated simultaneously to a value of 37±21 beats per minute when the duration of antecedent sympathetic stimulation was 10 minutes. Sympathetic stimulation releases not only norepinephrine but also neuropeptide Y, and this neuropeptide inhibits vagal neurotransmission. Our data suggest, therefore, that the longer the antecedent sympathetic stimulation, the greater the inhibition of vagal neurotransmission (presumably by the neuropeptide Y) and, therefore, the less pronounced the vagal predominance. (Circulation Research 1992;71:898–905)

KEY WORDS • acetylcholine • autonomic nervous system • heart rate • neuropeptide Y • norepinephrine • vagus nerves

The opposing effects of sympathetic and vagal activity on the heart do not summate algebraically, but complex interactions prevail.1–5 Early studies have revealed that when the neurons of both autonomic divisions are stimulated simultaneously, the vagal effects tend to predominate over the sympathetically effective effects on certain cardiac functions, notably heart rate.1–5

Traditionally, the neural control of the heart has been considered to be mediated mainly by norepinephrine (NE) released from sympathetic nerve endings and acetylcholine (ACh) released from vagal nerve endings.3–5 Hence, the autonomic interactions were believed to be mediated almost exclusively by these two neurotransmitters. More recent studies have disclosed, however, that many nonadrenergic, noncholinergic substances, including various neuropeptides, are released along with NE and ACh.6–9

With regard to the neural control of the heart, a number of studies have shown that an antecedent period of intense sympathetic stimulation can profoundly and persistently attenuate the chronotropic responses to subsequent vagal test stimulations.10–16 This profound inhibition of vagal efficacy by antecedent sympathetic activity is believed to be mediated by the release of a specific neuropeptide from the sympathetic nerve endings; in the dog, the specific neuromodulator is neuropeptide Y (NPY).10–16

When intense vagal stimulation is given concurrently with the sympathetic stimulation, however, the chronotropic responses to subsequent vagal test stimulations are no longer attenuated.15 Thus, a period of intense sympathetic activity alone would be expected to abolish or attenuate vagal preponderance for a substantial time after cessation of the antecedent sympathetic activity. Conversely, an equivalent period of concurrent intense vagal and sympathetic activity would be expected to have only a negligible influence on subsequent vagal preponderance. Hence, the temporal relations between periods of sympathetic and vagal activity would be expected to influence substantially the nature and magnitude of the interactions that take place between the two divisions of the autonomic nervous system.

The present experiments were designed to test the hypothesis that differences in the time of initiation of cardiac sympathetic and vagal activities is a critical determinant of the interaction between these two autonomic divisions. We compared the cardiac responses to

From the Department of Investigative Medicine, Mt. Sinai Medical Center, and Case Western Reserve University, Cleveland, Ohio.

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Address for correspondence: Matthew N. Levy, MD, Department of Investigative Medicine, Mt. Sinai Medical Center, 1 Mt. Sinai Dr., Cleveland, OH 44106.

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the following two patterns of concurrent sympathetic and vagal stimulation: one in which the sympathetic activation preceded the vagal activation and the other in which the order was reversed (Figure 1). In both patterns, the sympathetic and vagal stimulations were given concurrently during the last minute of the combined stimulation, and both stimulations were discontinued simultaneously.

Materials and Methods

Surgical Preparations

Sixteen mongrel dogs (8–24 kg) were premedicated with morphine sulfate (2 mg/kg i.m.) and anesthetized with α-chloralose (100 mg/kg i.v.). A tracheal cannula was inserted through a midline cervical incision. The chest was opened transversely at the fourth intercostal space, and intermittent positive-pressure ventilation was started via the tracheal cannula. The pericardium was then opened. The atrial electrogram (A wave) was recorded from a bipolar electrode catheter introduced into the right atrial appendage. The cardiac cycle length (AA interval) was calculated from the atrial electrogram by an analog computer (model 580, Electronic Associates Inc., West Long Beach, N.J.). The atrial electrogram, the AA interval, and the arterial blood pressure were recorded on an eight-channel oscillograph (model ES 1000, Gould, Cleveland, Ohio). Arterial blood pressure was recorded from a femoral artery by means of a Statham transducer (model P23BB, Gould).

Both cervical vagi were crushed with ligatures at the midcervical level to interrupt neural conduction. A pair of stainless-steel plunge electrodes (0.2 mm, insulated to within 1 mm of the tip) was inserted into the right vagus nerve caudal to the ligature. The electrodes were connected to an electronic stimulator (model SD-9, with isolation unit, Grass Instrument Co., Quincy, Mass.). Stimulation through such plunge electrodes evokes stable responses over many hours.17 When vagal stimulation blocked atrioventricular (AV) conduction, we paced the right ventricle by a second Grass stimulator to maintain the same heart rate that prevailed before vagal stimulation.

The right and left stellate ganglia were isolated. The upper poles of both stellate ganglia were crushed by tight ligatures to interrupt the tonic sympathetic impulses to the heart. Shielded iridium electrodes (Harvard Apparatus, South Natick, Mass.) were applied to the right ansa subclavia only and were connected to a third Grass stimulator.

Stimulation Protocol

We stimulated the cardiac sympathetic and vagus nerves supramaximally (10–15 V, 1 msec in duration) at
a frequency of 10 Hz under two sets of activation patterns (Figure 1). Animals were subjected randomly to one or the other of these stimulation patterns; each pattern was used in eight animals.

In activation pattern SV (Figure 1A), the duration of vagal stimulation was always 1 minute. During one of the stimulation periods, the vagus nerve alone was stimulated (SnV1). When sympathetic and vagus nerves were both stimulated, the following time differences prevailed between the beginnings of sympathetic and vagal stimulation: sympathetic preceded vagal stimulation by 0 minutes (SnV1), 2.5 minutes (SnV1), 5 minutes (SnV1), and 10 minutes (SnV1). To enable us to evaluate the autonomic interactions, we also stimulated the sympathetic nerve alone with train durations of 1 minute (SnV1), 3.5 minutes (SnV1), 6 minutes (SnV1), and 11 minutes (SnV1).

In activation pattern VS (Figure 1B), the duration of sympathetic stimulation was always 1 minute. During one period, the sympathetic nerve alone was stimulated (VnS1). When the vagus and sympathetic nerves were both stimulated, the following time differences prevailed between the beginning of vagal and sympathetic stimulation: vagal preceded sympathetic stimulation by 0 minutes (V1S1), 2.5 minutes (V1S1), 5 minutes (V1S1), and 10 minutes (V1S1). We also stimulated the vagus nerve alone with train durations of 1 minute (V1S0), 3.5 minutes (V1S0), 6 minutes (V1S0), and 11 minutes (V1S0).

In each animal, regardless of whether we used pattern SV or VS, we randomized the sequence of application of the various stimulation combinations. After each stimulation combination, we allowed the animal to recover almost completely (by approximately 95%) before we applied the next stimulation combination. To assess the extent of recovery, we determined periodically the chronotropic responses to test vagal stimulations (each 5 Hz for 1 minute); such responses serve as a sensitive index of the persistence of the effects of neurally released NPY on vagal neurotransmission. Particularly after the longer trains of sympathetic stimulation that we used in pattern SV, the inhibitory effects on vagal neurotransmission (presumably ascribable to NPY) could be detected for approximately 45 minutes. We did not apply any stimulation combination until the response to the test vagal stimulations delivered after the preceding stimulation combination had recovered by approximately 95%.

Because of the long-lasting actions of NPY and the consequent irregularities in the times between the random applications of the various stimulation combinations, the actual sequence used in a given animal was a significant determinant of the responses obtained in that animal. However, because we did randomize the application sequence in each animal, this factor was minimized for the composite data derived for each group of animals.

Data Analysis

The chronotropic responses to various combinations of vagal and sympathetic stimulations were analyzed by means of a multifactorial mixed-model analysis of variance. We considered \( p \leq 0.05 \) to be statistically significant.

Results

Representative Experiment

Figure 2 shows the changes in the AA interval obtained by three of the neural stimulations included in activation pattern SV in a representative experiment. The basic cardiac cycle length (AA interval) was 540 msec; i.e., the heart rate was 111 beats per minute. Vagal stimulation alone (SnV1) evoked a steady-state increase in the AA interval of approximately 1,030 msec.
Concurrent sympathetic and vagal stimulations \((S_1V_1)\) for 1 minute produced a steady-state increase in the AA interval of approximately 790 msec (Figure 2B). When sympathetic stimulation preceded the vagal stimulation by 5 minutes \((S_1V_1, \text{Figure 2C})\), the initial period of sympathetic stimulation decreased the AA interval by approximately 260 msec. Concurrent vagal stimulation during the last minute evoked a steady-state increase in the AA interval of approximately 525 msec. Thus, combined sympathetic and vagal stimulation elicited a much smaller increase in cycle length when sympathetic stimulation preceded combined stimulation by 5 minutes \((\Delta AA, 525 \text{ msec})\) than when sympathetic and vagal stimulation began simultaneously \((\Delta AA, 790 \text{ msec})\). Note that the vertical scale for panel C is different from that for panels A and B in Figure 2.

Figure 3 shows the chronotropic responses evoked by all the pattern SV stimulation combinations in the same animal from which Figure 2 was derived. The chronotropic responses have been expressed in terms of heart rate rather than cardiac cycle length, to make the analysis of the autonomic interactions consistent with our previously published analyses of cardiac autonomic interactions.\(^{1,2-5}\) The 1-minute period of sympathetic stimulation alone \((S_1V_0)\) increased the heart rate by 98 beats per minute (curve \(V_0\) in Figure 3A). The longer trains (i.e., \(S_3V_0\), \(S_6V_0\), and \(S_1(V_0)\) of sympathetic stimulation all increased the heart rate by 94 beats per minute (curve \(V_0\)). The 1-minute period of vagal stimulation alone \((S_1V_1)\) decreased the heart rate by 73 beats per minute (curve \(V_1\)). Concurrent vagal and sympathetic stimulation \((S_1V_1)\), each for 1 minute, diminished the heart rate by 69 beats per minute (curve \(V_1\)). Thus, combined vagal and sympathetic stimulation decreased heart rate almost as much as did vagal stimulation alone, despite the fact that sympathetic stimulation \((S_1V_0)\) alone, at this same level, increased heart rate by almost 100 beats per minute (curve \(V_0\)).

This predominance of the vagal over the sympathetic effects on heart rate became less pronounced as the sympathetic stimulations preceded the vagal stimulations by progressively more time. When the sympathetic stimulation preceded vagal stimulation by 2.5 minutes \((S_3V_1)\), 5 minutes \((S_6V_1)\), and 10 minutes \((S_1V_1)\), the reductions in heart rate evoked by the combined stimulations progressively diminished (curve \(V_1\), Figure 3). The effects of vagal stimulation during the various concurrent stimulations are represented by the vertical differences between curve \(V_1\) and \(V_0\) in Figure 3A. These differences are plotted as vertical bars in Figure 3B.

**Composite Data**

Figure 4 shows the mean chronotropic responses to activation pattern SV in a group of eight dogs. Note that vagal stimulation alone \((S_1V_1)\) for 1 minute decreased heart rate by 64 beats per minute (curve \(V_1\)), whereas sympathetic stimulation alone \((S_1V_0)\) for 1 minute increased heart rate by 100 beats per minute (curve \(V_0\)). Even though sympathetic stimulation increased heart rate more than vagal stimulation decreased it, simultaneous sympathetic and vagal stimulation \((S_1V_1)\) for 1 minute actually decreased the heart rate by 31 beats per minute (curve \(V_1\)). Thus, the vagal effects predominated over the sympathetic effects. This nonlinear summation (interaction) of the sympathetic and vagal effects has been termed "accentuated antagonism."\(^{10}\) Analysis of variance showed not only that the individual sympathetic \((S_1V_0)\) and vagal \((S_1V_1)\) effects were highly significant \((p<0.001)\) but also that the interaction between the sympathetic and vagal effects was highly significant \((p<0.001)\).

To determine whether the elapsed time between the beginnings of sympathetic and vagal stimulation affected the vagal preponderance, we compared the chronotropic responses evoked when the sympathetic stim-
ulation preceded vagal stimulation by 0, 2.5, 5, and 10 minutes (curve V1, Figure 4A) with the responses evoked by the sympathetic stimulations alone (curve V0, Figure 4A). Figure 4B displays the differences between the ordinate values for curves V1 and V0 for the various stimulation combinations. The height of each bar above the horizontal dashed line is an index of the accentuated antagonism. For example, vagal stimulation alone (S0V0) for 1 minute decreased heart rate by 64 beats per minute. However, in the presence of concurrent sympathetic stimulation for 1 minute, the same vagal stimulation (S1V0) was responsible for a much greater reduction in heart rate (131 beats per minute). Thus, the inhibitory effect of the vagal stimulation was 67 beats per minute greater in the presence than in the absence of sympathetic stimulation. However, the accentuated antagonism diminished (p=0.01) as the elapsed time between the beginnings of sympathetic and vagal stim-

ulation increased, as reflected by the different heights of the bars above the horizontal dashed line in Figure 4B.

Figure 5 shows the mean chronotropic responses to activation pattern VS in a second group of eight dogs. The mean increase in heart rate elicited by sympathetic stimulation alone (V0S1) for 1 minute was 83 beats per minute (curve S1), and the mean decrease elicited by vagal stimulation (V0S0) alone for 1 minute was 70 beats per minute (curve S0). Although the sympathetically induced increment in heart rate exceeded the vagally induced decrement by 13 beats per minute, simultaneous stimulation of the two autonomic divisions for 1 minute (V1S1) actually decreased the heart rate by 39 beats per minute (curve S1). Thus, the vagal effects on heart rate predominated over the sympathetic effects, just as they did in the preceding group of experiments. Analysis of variance showed that the heart rate was significantly affected (p<0.001) by individual sympa-
thetic (V₃S₃) and vagal (V₅S₅) stimulation and that the interaction between vagal and sympathetic effects was also highly significant (p<0.001).

To determine whether the elapsed time between the beginnings of vagal and sympathetic stimulation influenced the vagal preponderance, we compared the chronotropic responses that were elicited when the vagal stimulation preceded sympathetic stimulation by 0, 2.5, 5, and 10 minutes (curve S₅) with the responses evoked by the vagal stimulations alone (curve S₅). Figure 5B displays the differences between the ordinate values for curves S₅ and S₅ for the various stimulation combinations. Note that sympathetic stimulation was responsible for a much greater increment in heart rate in the absence of vagal stimulation (V₃) than in its presence (V₅V₅); this manifests the vagal preponderance. The distance from the top of each bar to the horizontal dashed line in Figure 5B is an index of the accentuated antagonism. When we began the vagal stimulations before the sympathetic stimulation (V₃SV₃), the vagal preponderance was slightly less pronounced than when the vagal and sympathetic stimulations began simultaneously (V₅); however, these differences were not significant (p=0.8).

Discussion

Postganglionic sympathetic and vagus nerve fibers often lie side by side in the walls of the heart. Therefore, the neurotransmitters and neuromodulators released from nerve fibers of one autonomic division can influence the release of transmitters from the nerve endings of the other division. This mutual modulation of neurotransmitter release by the two autonomic divisions is a cardinal factor responsible for the complex interactions that prevail in the autonomic regulation of the heart.

A prominent feature of such cardiac autonomic interactions is that the vagal effects tend to predominate over the sympathetic effects, especially with respect to the control of heart rate. A previous study from our laboratory showed that the positive chronotropic effect of a given level of cardiac sympathetic stimulation was progressively attenuated as we raised the level of concurrent vagal activity. This nonlinear summation represents a characteristic example of accentuated antagonism. Such vagal preponderance can be ascribed partly to the tendency for the vagally released ACh to inhibit the release of NE from nearby sympathetic nerve endings.

Our present data confirm the vagal preponderance that prevails in the control of heart rate when the fibers from the two autonomic divisions are stimulated simultaneously. With all of the stimulation combinations used in the present experiments, the vagal effects on heart rate predominated over the sympathetic effects. In the experiments depicted in Figure 4, for example, vagal stimulation alone decreased the heart rate by approximately 65 beats per minute. However, in the presence of concurrent sympathetic stimulation, the identical vagal stimulation accounted for a reduction in heart rate of approximately 130 beats per minute.

Our experiments also showed that temporal differences between the beginnings of sympathetic and vagal activity substantially affected the sympathetic–vagal interaction. When sympathetic activation preceded vagal activation, the vagal predominance diminished as we increased the time between the beginnings of the sympathetic and vagal activations (Figures 3 and 4). However, when vagal activation preceded sympathetic activation, the vagal predominance was not significantly affected by the elapsed time between the beginnings of vagal and sympathetic activation (Figure 5).

Our results are compatible with the recent findings of Revington and McCloskey, who compared the effects of sympathetic stimulation alone, vagal stimulation alone, and combined sympathetic and vagal stimulation on the chronotropic responses to subsequent vagal test stimulations. They found that intense vagal stimulation alone actually potentiated the chronotropic responses to subsequent vagal test stimulations. They also found that intense sympathetic stimulation alone profoundly and persistently attenuated the chronotropic responses to subsequent vagal test stimulations. Our experimental results (Figure 4) confirm this prediction.

The mechanism by which intense sympathetic stimulation attenuates the chronotropic responses to subsequent vagal stimulation appears to involve the inhibition of ACh release from vagal nerve endings by the NPY released from the sympathetic nerve endings. Although the principal evidence for the inhibition of ACh release by neurally released NPY is indirect, it is very convincing: 1) Intense sympathetic stimulation persistently inhibits the chronotropic responses to subsequent vagal test stimulations. 2) Sympathetic excitation releases both NE and NPY. 3) Exogenous NPY, but not NE, mimics the effects of intense sympathetic stimulation on the chronotropic responses to subsequent vagal test stimulations. 4) Neither intense sympathetic stimulation nor exogenous NPY alters the subsequent chronotropic responses to muscarinic agonists. Experiments on isolated colon preparations support the hypothesis that NPY inhibits parasym pathetic neurotransmission by inhibiting the release of ACh. In such preparations, NPY was found to inhibit the K⁺-evoked release of ACh.

When we used activation pattern SV in the present study, sympathetic stimulation preceded vagal stimulation by 0–10 minutes. We found that the vagal predominance (measured near the end of the 1-minute period of concurrent stimulation) was diminished as we increased the elapsed time between the beginnings of sympathetic and vagal stimulation (Figure 4). We be-
lieve that as we prolonged this period of antecedent sympathetic stimulation, the cumulative amount of NPY released from the sympathetic terminals into the cardiac interstitium increased. The greater quantity of released NPY probably inhibited more profoundly the release of ACh from the vagal terminals. Hence, the vagal preponderance was diminished during the final period of concurrent sympathetic and vagal stimulation.

A previous study from our laboratory showed that the magnitude and duration of the inhibition of vagal neurotransmission were augmented as we increased the duration of an antecedent period of intense sympathetic stimulation. Another study from our laboratory has shown that the magnitude and duration of the inhibition of vagal neurotransmission correlate closely with the overflow of NPY into the coronary sinus blood. These studies support our contention that the release of NPY increases as the period of sympathetic stimulation is prolonged. Hence, the elapsed time between the beginnings of sympathetic and vagal activation appears to be a crucial determinant of the sympathetic–vagal interaction (Figure 4).

However, when we used activation pattern VS, the vagal predominance was not significantly affected by the elapsed time between the beginnings of sympathetic and vagal stimulation (Figure 5). In the experiments of Revington and McCloskey, when the sympathetic and vagus nerves were stimulated concurrently, the persistent attenuation of the subsequent chronotropic responses to vagal test stimuli were diminished or abolished. These investigators concluded that the concurrent vagal activity must have inhibited the release of NPY from the cardiac sympathetic nerve endings; it is the NPY that can persistently inhibit the cardiac responses to vagal test stimuli. However, Revington and McCloskey found that the effect of the vagal stimulation on the putative inhibition of NPY release was very brief; i.e., the effect of any given vagal stimulus pulse persisted for no more than 5–10 seconds. The transient nature of the vagal effect suggested that the inhibition of NPY was mediated by the vagal release of ACh, which does have a transient action, rather than by the vagal release of some neuropeptide, which would characteristically have a much longer action.

In the present experiments in which we used activation pattern VS (Figure 5), the vagal predominance was not affected significantly by the elapsed time between the beginnings of the vagal and sympathetic stimulations. The explanation probably resides in the transient nature of the ACh effect. If the ACh effect persists for no more than a few seconds, then changing the elapsed time between the beginnings of vagal and sympathetic stimulation from 10 seconds to 10 minutes would not be expected to have an appreciable influence.

Thus, the disparate effects of the sequence of initiation of sympathetic and vagal activity (as exemplified by activation patterns SV and VS) on the autonomic interactions are probably attributable to the different durations of actions of the classical transmitters (ACh and NE) and of the neuropeptides (e.g., NPY). When vagal activity begins before sympathetic activity, the vagal endings release ACh, which effectively, but only transiently, inhibits NE release from neighboring sympathetic terminals. Conversely, when sympathetic activity begins before vagal activity, the sympathetic termi-


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