Cardiac sympathetic nerve stimulation does not attenuate dynamic vagal control of heart rate via $\alpha$-adrenergic mechanism

Tadayoshi Miyamoto, Toru Kawada, Yusuke Yanagiya, Masashi Inagaki, Hiroshi Takaki, Masaru Sugimachi, and Kenji Sunagawa

1Department of Cardiovascular Dynamics, National Cardiovascular Center Research Institute, Osaka 565-8565; 2Japan Association for the Advancement of Medical Equipment, Tokyo 105-0013; and 3The Organization for Pharmaceutical Safety and Research, Tokyo 100-0013, Japan

Submitted 7 August 2003; accepted in final form 6 March 2004

Miyamoto, Tadayoshi, Toru Kawada, Yusuke Yanagiya, Masashi Inagaki, Hiroshi Takaki, Masaru Sugimachi, and Kenji Sunagawa. Cardiac sympathetic nerve stimulation does not attenuate dynamic vagal control of heart rate via $\alpha$-adrenergic mechanism. Am J Physiol Heart Circ Physiol 287: H860–H865, 2004. First published March 11, 2004; 10.1152/ajpheart.00752.2003.—Complex sympathovagal interactions govern heart rate (HR). Activation of the postjunctional $\beta$-adrenergic receptors on the sinus nodal cells augments the HR response to vagal stimulation, whereas exogenous activation of the presynaptic $\alpha$-adrenergic receptors on the vagal nerve terminals attenuates vagal control of HR. Whether the $\alpha$-adrenergic mechanism associated with cardiac postganglionic sympathetic nerve activation plays a significant role in modulation of the dynamic vagal control of HR remains unknown. The right vagal nerve was stimulated in seven anesthetized rabbits that had undergone sinoaortic denervation and vagotomy according to a binary white-noise signal (0–10 Hz) for 10 min; subsequently, the transfer function from vagal stimulation to HR was estimated. The effects of $\beta$-adrenergic blockade with propranolol (1 mg/kg iv) and the combined effects of $\beta$-adrenergic blockade and tonic cardiac sympathetic nerve stimulation at 5 Hz were examined. The transfer function from vagal stimulation to HR approximated a first-order, low-pass filter with pure delay. $\beta$-Adrenergic blockade increased the dynamic gain from $6.0 \pm 0.4$ to $3.7 \pm 0.6$ beats·min$^{-1}$·Hz$^{-1}$ ($P<0.01$) with no alteration of the corner frequency or pure delay. Under $\beta$-adrenergic blockade conditions, tonic sympathetic stimulation did not further change the dynamic gain (3.8 $\pm$ 0.5 beats·min$^{-1}$·Hz$^{-1}$). In conclusion, cardiac postganglionic sympathetic nerve stimulation did not affect the dynamic HR response to vagal stimulation via the $\alpha$-adrenergic mechanism.

systems analysis; transfer function; $\beta$-adrenergic blockade; rabbits; sympathovagal interaction

COMPLEX SYMPATHOVAGAL INTERACTIONS are known to occur in the regulation of heart rate (HR). These interactions involve neural interactions within and between cardiac ganglia (2, 3), at the end terminals for their cardiac projections (18), and via second-messenger systems in the innervated myocytes (29). An increase in background sympathetic tone augments the HR response to vagal nerve activity (19, 20). Levy (19) referred to this phenomenon as an accentuated antagonism of HR control. Accumulation of cAMP in the sinus nodal cells via activation of postjunctional $\beta$-adrenergic receptors contributed to the accentuated antagonism (25). On the other hand, activation of the prejunctional $\alpha$-adrenergic receptors attenuated ACh release from the cardiac vagal nerve terminals (1, 22, 23, 26–28, 30, 31). Akiyama et al. (1) demonstrated that local norepinephrine (NE) administration in the feline heart attenuated myocardial interstitial ACh release during electrical vagal stimulation via the $\alpha$-adrenergic mechanism. These findings, in concert, indicate that whether the HR response to vagal stimulation is augmented or attenuated by concomitant sympathetic tone depends on the type and site of adrenergic receptors most selectively activated under a given condition.

Our previous studies performed on the rabbit indicated that elevated cardiac sympathetic nerve activity augmented the dynamic HR response to vagal nerve stimulation via activation of the postjunctional $\beta$-adrenergic cascade (13, 14, 25). On the other hand, high plasma NE levels attenuated the dynamic HR response to vagal stimulation via the $\alpha$-adrenergic mechanism (24). However, whether NE released from cardiac postganglionic sympathetic nerve terminals exerts presynaptic inhibition on vagal control of HR via the $\alpha$-adrenergic mechanism remains unknown. We hypothesized that cardiac postganglionic sympathetic nerve stimulation under $\beta$-adrenergic blockade conditions would manifest, if any, the presynaptic inhibition on vagal control of HR. The objective of the present investigation was therefore to examine the effects of tonic sympathetic nerve stimulation on the dynamic vagal control of HR under $\beta$-adrenergic blockade conditions. The results indicated that cardiac postganglionic sympathetic nerve stimulation did not affect the dynamic HR response to vagal stimulation via the $\alpha$-adrenergic mechanism.

MATERIALS AND METHODS

Surgical preparations. Animal care was in accordance with “Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences,” which was approved by the Physiological Society of Japan. Twelve Japanese White rabbits (body wt, 2.5–3.1 kg) were anesthetized via injection (2 ml/kg iv) of a mixture of urethane (250 mg/ml) and $\alpha$-chloralose (40 mg/ml); subsequently, the rabbits were mechanically ventilated with oxygen-enriched room air. Supplemental doses of these anesthetics were administered as necessary via the marginal ear vein. Aortic pressure (AP) was monitored with a micromanometer catheter (Millar Instruments; Houston, TX) inserted via the right femoral artery. A catheter for drug administration was inserted into the right femoral vein. Sinoaortic denervation was performed bilaterally to minimize changes in sympathetic efferent nerve activity via the arterial baroreflexes. The vagi were sectioned bilaterally at the neck. A pair of bipolar platinum electrodes...
was then attached to the cardiac end of the sectioned right vagus for dynamic vagal stimulation. The right inferior cardiac sympathetic nerve, which mainly consists of postganglionic nerve fibers in the rabbit (15), was exposed through a midline thoracotomy. A second pair of bipolar platinum electrodes was attached for tonic sympathetic nerve stimulation. To prevent drying and provide insulation, the stimulation electrodes and the nerves were immersed in a mixture of white petroleum jelly (Vaseline) and liquid paraffin. Instantaneous HR was measured from the AP signal utilizing a cardiotachometer (model N4778, San-ei). Body temperature was maintained at 38°C with a heating pad throughout the experiment.

**Protocols.** The pulse duration of nerve stimulation was set at 2 ms. The stimulation amplitude of the right vagus was adjusted to yield a HR decrease of ∼50 beats/min at a stimulation frequency of 5 Hz. After adjustment, the amplitude of vagal stimulation ranged from 3.5 to 6.0 V. The stimulation amplitude of the right cardiac postganglionic sympathetic nerve was adjusted to yield an HR increase of 50 beats/min at a stimulation frequency of 5 Hz. After adjustment, the amplitude of sympathetic stimulation ranged from 1.8 to 3.8 V.

In protocol 1 (n = 7), which was done to estimate the transfer function from vagal stimulation to HR response, the right vagus was stimulated employing a frequency-modulated pulse train. The stimulation frequency was switched every second at either 0 or 10 Hz according to a binary white-noise signal. The power spectrum of the stimulation signal, which was reasonably constant up to 0.5 Hz, decreased gradually to 1/10 at ∼0.8 Hz and diminished sharply as the frequency increased to 1 Hz. The transfer function was estimated only up to 0.8 Hz, because the reliability of estimation decreased due to the lack of input power above this frequency. The selected frequency range sufficiently spanned the physiological range of interest with respect to dynamic vagal control of HR in rabbits (13, 14, 24, 25).

The transfer function from dynamic vagal stimulation to the HR response was estimated from 10-min data under the following three conditions: control, β-adrenergic blockade, and β-adrenergic block- ade plus tonic sympathetic nerve stimulation. After the control data was recorded, a bolus injection of propranolol (1 mg/kg iv) was administered; ∼10 min elapsed before HR and AP reached the new steady state. Under β-adrenergic blockade conditions, estimation of the transfer function was repeated with and without simultaneous 5-Hz tonic sympathetic stimulation. The order of the latter two conditions was randomized across the animals. The control data was obtained first in all animals, as the long-lasting effects of propranolol did not permit the subsequent acquisition of control data.

In protocol 2 (n = 5), which was done to examine the stability of electrical sympathetic stimulation, 1-min stimulation of the right cardiac postganglionic sympathetic nerve was repeated every 10 min for 6 stimulations.

**Data analysis.** Data were digitized at 200 Hz utilizing a 12-bit analog-to-digital converter and stored on the hard disk of a dedicated laboratory computer system.

In protocol 1, prestimulation values for HR and AP were obtained by averaging the respective data for 10 s immediately before vagal stimulation in each condition. Mean values for HR and AP during vagal stimulation were calculated by averaging the respective data over the time period of dynamic vagal stimulation.

The transfer function from dynamic vagal stimulation to the HR response was estimated based on the following procedure. Input-output data pairs of the vagal stimulation frequency and HR were resampled at 10 Hz; subsequently, data pairs were partitioned into eight 50%-overlapping segments consisting of 1,024 data points each. For each segment, the linear trend was subtracted and a Hanning window was applied. A fast Fourier transform was then performed to obtain the frequency spectra for vagal stimulation [S(\(f\))] and HR [HR(\(f\))] (7). Over the eight segments, the power of the vagal stimulation [\(S_N(\omega_0)\)], that of HR [HR(\(f\))] and the cross-power between these two signals [\(S_{N\cdotHR}(\omega_0)\)] were ensemble averaged. Finally, the transfer function [\(H(\omega_0)\)] from vagal stimulation to HR response was determined using the following equation (4, 21)

\[
H(\omega_0) = \frac{S_{N\cdotHR}(\omega_0)}{N S_N(\omega_0)}
\]

The transfer function from vagal stimulation to HR response approximated a first-order, low-pass filter with pure delay in previous studies (13, 14, 24, 25); therefore, the estimated transfer function was parameterized with the equation

\[
H(\omega_0) = \frac{-K}{1 + \frac{\omega_0}{f_c}} e^{-2\pi j L}
\]

where \(K\) represents dynamic gain (in \(\text{beats} \cdot \text{min}^{-1} \cdot \text{Hz}^{-1}\), \(f_c\) denotes corner frequency (in Hz), \(L\) denotes pure delay (in s), and \(f\) and \(j\) represent frequency and an imaginary unit, respectively. The negative sign in the numerator indicates the negative HR response to vagal stimulation. The parameters were estimated by means of iterative nonlinear least-squares regression.

To quantify the linear dependence of the HR response on vagal stimulation, a nonlinear least-squares regression was performed to estimate the structural parameters of the following equation (4, 21)

\[
HR(t) = K(\omega_0) e^{-2\pi j L} + C
\]

where \(K(\omega_0)\) represents dynamic gain (in \(\text{beats} \cdot \text{min}^{-1} \cdot \text{Hz}^{-1}\)), \(C\) denotes the baseline (in beats/min), \(\omega_0\) denotes frequency (in Hz), and \(L\) denotes pure delay (in s).
stimulation, the magnitude-squared coherence function $Coh(f)$ was estimated employing the equation (4, 21)

$$ Coh(f) = \frac{|S_{V-HR}(f)|^2}{S_{N-HR}(f) \cdot S_{N-V}(f)} $$

Coherence value ranges from zero to unity. Unity coherence indicates perfect linear dependence between the input and output signals; in contrast, zero coherence indicates total independence between the two signals.

In protocol 2, pretreatment values of HR and AP were obtained by averaging the respective data for 10 s immediately before each 1-min sympathetic stimulation. The steady-state HR value was obtained by averaging instantaneous HR data for the last 10 s of each 1-min sympathetic stimulation. The HR increase at each sympathetic stimulation was calculated from the difference between the steady-state and pretreatment HR values.

Statistics. All data are presented as means ± SE. In protocol 1, the mean levels of HR and AP and fitted parameters of the transfer function were compared among conditions of control, β-adrenergic blockade, and β-adrenergic blockade plus tonic sympathetic stimulation by repeated-measures ANOVA. When a significant difference ($P < 0.05$) was evident among the three conditions, Tukey’s test for all pairwise comparisons (9) was applied to identify the inequality between the two conditions with a significance level of $P < 0.05$.

Results

Figure 1 exhibits typical recordings of vagal stimulation and HR response under conditions of control, β-adrenergic blockade, and β-adrenergic blockade plus tonic sympathetic nerve stimulation. Random vagal stimulation decreased HR intermittently. β-Adrenergic blockade decreased mean HR and attenuated the amplitude of HR variation. Tonic sympathetic nerve stimulation in the presence of the β-adrenergic blockade did not further affect the amplitude of HR variation. The speed of the response of HR to vagal stimulation appeared to be unchanged across the three conditions.

Table 1 summarizes pretreatment and mean values of HR and AP during vagal stimulation averaged from all animals in protocol 1. β-Adrenergic blockade significantly decreased pretreatment and mean values of HR during vagal stimulation. Tonic sympathetic nerve stimulation did not alter pretreatment and mean values of HR compared with the β-adrenergic blockade conditions. Changes in AP were not statistically significant.

Figure 2 illustrates the transfer functions from vagal stimulation to the HR response averaged from all animals in protocol 1. The gain plots, phase plots, and coherence functions are shown. The transfer gain was relatively constant below 0.05 Hz and decreased above 0.05 Hz up to 0.8 Hz in each panel. The phase approached $-\pi$ radians at the lowest frequency and lagged with increasing frequency. Coherence was near unity in the frequency range below 0.5 Hz under control conditions. A slight decrease from unity in the coherence values was noted under β-adrenergic blockade conditions with and without tonic sympathetic nerve stimulation.

The fitted parameters of the transfer functions are summarized in Table 2. β-Adrenergic blockade decreased dynamic gain with no alteration of the corner frequency or pure delay. Under β-adrenergic blockade conditions, tonic sympathetic nerve stimulation did not further change the dynamic gain.

Table 3 shows the pretreatment values of HR and AP and the HR increase in response to tonic sympathetic nerve

Table 1. Mean heart rate and aortic pressure values before and during vagal stimulation obtained from protocol 1

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>β-Blockade</th>
<th>β-Blockade + SS</th>
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<tbody>
<tr>
<td>Heart rate, beats/min</td>
<td>243±19</td>
<td>206±12*</td>
<td>204±11*</td>
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<tr>
<td>Prestimulation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>During stimulation</td>
<td>208±16</td>
<td>185±12**</td>
<td>184±12**</td>
</tr>
<tr>
<td>Aortic pressure, mmHg</td>
<td>103±6</td>
<td>90±6</td>
<td>89±9</td>
</tr>
<tr>
<td>Prestimulation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>During stimulation</td>
<td>101±7</td>
<td>88±6</td>
<td>89±7</td>
</tr>
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</table>

Values are means ± SE. β-Blockade, β-adrenergic blockade with propranolol (1 mg/kg iv); β-blockade + SS, β-adrenergic blockade with propranolol and constant cardiac sympathetic stimulation at 5 Hz. *$P < 0.01$; **$P < 0.05$ vs. corresponding control.
stimulation obtained from protocol 2. The HR increase to tonic sympathetic nerve stimulation did not change significantly for 50 min, which covered the entire time period of protocol 1.

**DISCUSSION**

We have demonstrated that β-adrenergic blockade with intravenous propranolol administration decreased the dynamic gain of the transfer function from vagal stimulation to HR. Under β-adrenergic blockade conditions, tonic stimulation of the cardiac postganglionic sympathetic nerve did not affect the dynamic gain of the HR response to vagal stimulation.

**Effects of tonic sympathetic nerve stimulation on dynamic vagal control of HR under β-adrenergic blockade conditions.**

Previous studies indicate that activation of α-adrenergic receptors modulates the vagal control of HR. Pardini et al. (26) showed that α-adrenergic stimulation by intravenous phenylephrine inhibited the HR response to electrical stimulation of the preganglionic vagal fibers, whereas this treatment facilitated the HR response to carbachol-induced activation of the postganglionic vagal fibers. In a previous investigation (24), we demonstrated that intravenous NE infusion attenuated dynamic vagal control of HR via the β-adrenergic mechanism. However, because these previous studies employed phenylephrine or NE administration, whether endogenous NE released from the cardiac sympathetic nerve terminals modulated the vagal control of HR via the α-adrenergic mechanism remained unknown.

In the present investigation, the effects of electrical stimulation of the cardiac postganglionic sympathetic nerve on vagal control of HR were examined under β-adrenergic blockade conditions. Selective stimulation of the cardiac sympathetic nerve did not increase plasma NE concentration perceptibly (8, 16, 32). Therefore, changes in the vagal control of HR, if any, could be attributable to the effects of NE released from the postganglionic cardiac sympathetic nerve terminals. As depicted in Fig. 2, dynamic vagal control of HR was unaffected by tonic sympathetic nerve stimulation under β-adrenergic blockade conditions, which suggests that modulation of the vagal control of HR via the α-adrenergic mechanism is negligible during selective stimulation of the cardiac sympathetic nerve. A failure to stimulate the sympathetic nerve cannot account for this observation, because the HR response to sympathetic nerve stimulation was reproducible in protocol 2 for the time period necessary for completing protocol 1 (see Table 3).

In addition to classical nicotinic cholinergic synapses, β-adrenergic synapses are present in sympathetic ganglia (3). Hence, β-adrenergic blockade can alter sympathetic transmission in the intrathoracic cardiac nervous system. However, because the right inferior sympathetic nerve we stimulated was mainly postganglionic in rabbits (15), the lack of sympathetic effects after β-adrenergic blockade cannot be ascribed to the interruption of sympathetic ganglionic transmission to the heart. Another factor that should be taken into account is the extent of overlap in innervation between the sympathetic and vagal systems. If the innervation does not overlap at all, NE released from the sympathetic nerve terminals may not reach the prejunctional α-adrenergic receptors on the vagal nerve terminals. However, we stimulated right-sided inputs for both parasympathetic and sympathetic inputs and thereby maximized the potential of neural interactions. Under similar experimental conditions, we detected sympathovagal interactions in the absence of β-adrenergic blockade in previous studies (13, 14). Therefore, we speculate that there was substantial overlap in innervation between the nerves we were activating.

**Effects of β-adrenergic blockade on dynamic vagal control of HR.**

The present results indicated that β-adrenergic blockade significantly attenuated the dynamic gain of the vagal control of HR (see Fig. 2). These results are consistent with findings in a previous study on dogs (6). β-Adrenergic blockade decreased mean HR during dynamic vagal stimulation (see Table 1), which suggests that a considerable sympathetic tone had existed under control conditions. Although the right inferior cardiac sympathetic nerve was sectioned, other sympathetic branches directed to the heart were kept intact and may have provided sympathetic tone under control conditions. The withdrawal of such background sympathetic tone after β-adrenergic blockade reduced the dynamic gain of the vagal control of HR.

Plasma catecholamines also participate in the background sympathetic tone. Although we demonstrated that high plasma NE concentration attenuated dynamic vagal control of HR (24), the effects of plasma epinephrine on dynamic vagal control of HR remain unknown. It is possible that plasma epinephrine exerted a positive chronotropic effect and augmented dynamic vagal control of HR under control conditions. However, previous studies using cardiac microdialysis have indicated that plasma catecholamines can dissociate from myocardial interstitial catecholamine levels due to substantial compartmentalization (16, 18). Myocardial interstitial NE levels correlate with left ventricular contractility regardless of

<table>
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<tr>
<th>Time, min</th>
<th>1</th>
<th>11</th>
<th>21</th>
<th>31</th>
<th>41</th>
<th>51</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔHR, beats/min</td>
<td>49.9±7.0</td>
<td>51.0±5.5</td>
<td>49.1±3.4</td>
<td>43.5±3.7</td>
<td>45.0±4.6</td>
<td>45.4±5.1</td>
</tr>
<tr>
<td>Prestimulation HR, beats/min</td>
<td>240.9±13.2</td>
<td>238.3±12.9</td>
<td>234.8±11.3</td>
<td>235.3±11.8</td>
<td>231.8±11.7</td>
<td>232.1±12.7</td>
</tr>
<tr>
<td>Prestimulation AP, mmHg</td>
<td>88.2±8.7</td>
<td>86.5±8.5</td>
<td>82.7±8.7</td>
<td>85.1±9.1</td>
<td>86.0±9.4</td>
<td>87.7±9.0</td>
</tr>
</tbody>
</table>

Values are means ± SE. HR, heart rate; AP, aortic pressure.
whether the interstitial NE levels were increased endogenously with cardiac sympathetic stimulation or exogenously with intravenous NE infusion (16). Therefore, not only plasma but also myocardial interstitial catecholamine levels are important for determining cardiac function. Additional studies are necessary to determine whether high levels of plasma and/or myocardial interstitial epinephrine modulate the dynamic vagal control of HR.

Limitations. First, data were obtained from animals under anesthetized conditions. In the event that data had been obtained from conscious animals, the results may have been different. However, because we stimulated the efferent pathways of the sectioned vagal and cardiac sympathetic nerves, the effects of anesthesia on the central nervous system may not influence the present results much. Furthermore, because we stimulated the efferent pathways of the sectioned vagal and cardiac sympathetic nerves, the results may have been obtained from conscious animals, the results may have been different. However, because we compared the efferent control of HR among the three different conditions under the same anesthesia, it is fair to say that the tonic sympathetic stimulation did not affect the vagal control of HR via the α-adrenergic mechanism.

Second, rabbits may exhibit a different degree of sympathovagal interaction compared with the more commonly studied dogs. However, the HR response to autonomic nerve stimulation is similar between rabbits and dogs in various aspects. The HR response to vagal stimulation is faster than that to sympathetic stimulation (5, 13). The accentuated antagonism (10, 19) and presynaptic inhibition (11, 28) between the vagal and sympathetic nerves were observed in both species. The three-dimensional plot derived from the steady-state HR response to various combinations of vagal and sympathetic stimulation is similar for the two species (14, 19). We therefore believe that rabbits are as good as dogs for investigating the autonomic control of HR.

Finally, the stimulation pattern of binary white noise differs from the physiological discharge of the vagal nerve (17). Notably, the aphasic nature of binary white-noise stimulation relative to each R-R interval would mask the phase-dependent sensitivity of the HR response to nerve stimulation (12). Moreover, we stimulated the entire bundle of the vagal nerve simultaneously. Thus the dynamic HR response determined in the present study cannot account for possible regional differences in nerve function among the nerve fibers.

In conclusion, cardiac postganglionic sympathetic nerve stimulation did not affect the dynamic HR response to vagal stimulation via the α-adrenergic mechanism in rabbits. Presynaptic inhibition of the vagal control of HR might manifest itself upon activation of the presynaptic α-adrenergic receptors on the preganglionic and/or postganglionic vagal nerve terminals by circulatory agents.

GRANTS

This study was supported by Research Grants for Cardiovascular Diseases (11C-3 and 11C-7) from the Ministry of Health and Welfare of Japan; by a Health Sciences Research Grant for Advanced Medical Technology from the Ministry of Health and Welfare of Japan; by a Grant-in-Aid for Scientific Research (B-11694337, C-11680802, and C-11670730) and a Grant-in-Aid for Encouragement of Young Scientists (13770378) from the Ministry of Education, Science, Sports, and Culture of Japan; by Japan Science and Technology Research and Development for Applying Advanced Computational Science and Technology; and by the Program for Promotion of Fundamental Studies in Health Science from the Organization for Pharmaceutical Safety and Research.

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