Accumulation of cAMP augments dynamic vagal control of heart rate

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Recent investigations in our laboratory using a Gaussian white noise perturbation technique have shown that simultaneous sympathetic stimulation augmented the gain of the transfer function from vagal stimulation frequency to heart rate response. However, the mechanism of that augmentation remains to be elucidated. In this study, we examined in anesthetized rabbits how three pharmacological interventions known to cause intracellular accumulation of cAMP affected the transfer function. Isoproterenol (0.3 µg·kg−1·min−1 iv) increased the dynamic gain of transfer function from 7.12 ± 0.67 to 12.4 ± 1.21 beats·min−1·Hz−1 (P < 0.05) without changing the corner frequency or the lag time. Similar augmentations were observed when forskolin (5 µg·kg−1·min−1 iv) or theophylline (20 mg/kg iv) was administered under conditions of β-adrenergic blockade. These results suggest that the accumulation of cAMP at postjunctional effector sites contributes, at least in part, to the sympathetic augmentation of the dynamic vagal control of heart rate.

Materials and Methods

Surgical preparations. Animal care was in accordance with institutional guidelines. Twenty-one Japanese white rabbits weighing 2.4–3.0 kg were anesthetically induced using an initial dose of urethan (250 mg/kg iv) and a chloralose (40 mg/kg iv) and mechanically ventilated with oxygen-enriched room air. Supplemental doses of anesthetics were given as necessary via the right femoral vein. Aortic pressure was monitored by means of a micromanometer catheter (model PC-340, 3-Fr, Millar Instruments, Houston, TX) inserted via the left femoral artery. Another catheter was inserted into the right femoral vein for the administration of drugs. The carotid sinus nerves and aortic depressor nerves were cut bilaterally to eliminate the effects of the arterial baroreflex systems. We transected the bilateral sympathetic nerves at the level of the stellate ganglion to eliminate the possible interaction between the vagus and sympathetic nerves. Vagus nerves were sectioned bilaterally at the neck, where a pair of bipolar platinum electrodes was attached to the cardiac end of the sectioned right vagus nerve for stimulation. To prevent drying and to provide insulation, the stimulation electrodes and the nerve were immersed in a mixture of white petrolatum (Vaseline) and paraffin. Finally, a pair of bipolar stainless electrodes was sutured to the right atrium to record the electrocardiogram for monitoring of HR. During all experiments, body temperature was maintained at 37°C with a heating pad.

Experimental procedures. The pulse duration of vagal stimulation was set at 2 ms. We adjusted the amplitude of stimulation to yield a HR decrease of −50 beats/min at 5 Hz.

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This resulted in an amplitude of stimulation ranging from 2.0 to 5.0 V (3.4 ± 0.3 V). To estimate the transfer function from vagal stimulation frequency to HR response, we stimulated the vagus nerve using a pulse train that was frequency modulated by a band-limited Gaussian white noise (10, 11, 22). The main advantage of such a Gaussian white-noise approach is that it enables estimation of the unbiased linear input-output relation even in the presence of significant nonlinearities in the system (18). The instantaneous stimulation frequency was switched every second. The power spectrum, fairly constant up to 0.5 Hz, decreased gradually to 1/10 at ~0.8 Hz and attenuated sharply as the frequency increased to 1 Hz. We estimated the transfer function only up to 0.8 Hz because the lack of input power above that frequency made the estimation unreliable. The frequency range sufficiently spanned the physiological range of the vagal control of HR (10, 11). We used different perturbation command sequences of Gaussian white noise for different animals.

In the first series of experiments (n = 7), we examined the effects of the β-adrenoceptor agonist isoproterenol on the transfer function from vagal stimulation frequency to HR response. We chose the dose of isoproterenol that would increase HR to ~300 beats/min, in so doing mimicking the conditions of sympathetic nerve stimulation at 5 Hz used in our previous study (10).

In the second (n = 7) and third (n = 7) series of experiments, we examined the effects of an adenylyl cyclase activator, forskolin, and a phosphodiesterase (PDE) inhibitor, theophylline, on the transfer function. In these experiments, we treated the rabbits with propranolol (0.5 mg/kg iv) to avoid a possible interference from circulating catecholamines. We confirmed by means of a preliminary study that the dose of propranolol used abolished the HR response to sympathetic nerve stimulation at 5 Hz. The doses of forskolin and theophylline were selected so as to enable matching of their mean levels of HR before vagal stimulation with that of the isoproterenol infusion experiment (i.e., ~300 beats/min), respectively.

We stimulated the vagus nerve with a Gaussian white noise of 5 ± 2 (SD) Hz both in the absence and in the presence of pharmacological intervention. Dynamic vagal stimulation was started 15~20 min after the beginning of each drug administration. After reaching a steady-state in each stimulation protocol, we recorded both the stimulation frequency and HR for 10 min.

HR and vagal stimulation frequency were digitized at 200 Hz using a 12-bit analog-to-digital converter and stored on the hard disk of a dedicated laboratory computer system (NEC PC-98, Tokyo, Japan). We calculated the mean level of HR before vagal stimulation by averaging the instantaneous HR measured for the 10 s preceding stimulation. The mean level of HR during vagal stimulation was calculated by averaging instantaneous HR for a given time period (10 min).

Estimation of the transfer function. After applying an anti-aliasing filter, we resampled the input (nerve stimulation frequency)-output (HR) data pairs at 10 Hz, then segmented the data into eight 50%-overlapping segments of 1,024 data points each. For each segment, the linear trend was subtracted and a Hanning window was applied. We then performed the fast Fourier transformation to obtain the frequency spectrum of nerve stimulation frequency, \( N(f) \), and that of HR response, \( H(f) \). The resolution of the frequency was 0.01 Hz.

We ensemble averaged, over the eight segments, the power of the nerve stimulation, \( S_{N,N}(f) \), the HR response, \( S_{HR,HR}(f) \), and the cross power between them, \( S_{HR-NR}(f) \). Finally, we obtained the transfer function, \( H(f) \), from nerve stimulation frequency to HR response using the following equation

\[
H(f) = \frac{S_{HR-RR}(f)}{S_{N,N}(f)}
\]

The modulus, \( |H(f)| \), and phase shift, \( \phi(f) \), of the transfer function were derived from its real part, \( H_r(f) \), and imaginary part, \( H_i(f) \), with the following equations

\[
|H(f)| = \sqrt{H_r(f)^2 + H_i(f)^2}
\]

\[
\phi(f) = \tan^{-1} \left( \frac{H_i(f)}{H_r(f)} \right)
\]

The modulus indicates the relative amplitude of HR change per unit change of nerve stimulation frequency and is expressed in units of beats per minute per Hz (beats·min⁻¹·Hz⁻¹). We hereafter refer to the modulus as the gain of the transfer function. The phase shift indicates, with respect to the input, a lag or lead of the output normalized by the corresponding frequency.

Because the transfer function from vagal stimulation frequency to HR response approximated a first-order low-pass filter with time lag (10), we parameterized the transfer function by using the following equations

\[
H(f) = \left[ \frac{-K}{1 + \left( \frac{f}{f_c} \right)^j} \right] e^{2\pi fjL}
\]

\[
j = \sqrt{-1}
\]

where \( K \) is the gain at ultralow frequencies (referred to hereafter as dynamic gain), \( f \) is frequency, \( f_c \) is the corner frequency, i.e., the frequency at which the gain decreases 3 dB from its steady-state value, and \( L \) is the time lag. The negative sign of \( K \) indicates the negative HR response to vagal stimulation.

To quantify the linear dependence of the HR response on nerve stimulation, we estimated the coherence function, \( \text{Coh}(f) \), by using the following equation

\[
\text{Coh}(f) = \frac{|S_{HR-RR}(f)|^2}{S_{N,N}(f)S_{HR-RR}(f)}
\]

After estimating the transfer functions, we calculated via inverse Fourier transformation the corresponding step responses in HR to a 1-Hz input nerve stimulation to easily visualize the system characteristics. The durations of the calculated step responses were 51.2 s. We derived maximum step responses by averaging the last 10 s of the calculated step responses. The time constants of the step responses were defined as the time required to reach 63.2% of the maximum step responses.

Chemicals. The following drugs were used: dl-isoproterenol hydrochloride, forskolin, dl-propranolol hydrochloride, and theophylline (Wako Pure Chemical Industries, Osaka, Japan). Isoproterenol was dissolved in 0.9% NaCl solution containing 0.01% L-ascorbic acid. Forskolin was prepared in 0.9% NaCl solution containing 1% ethanol. Theophylline was prepared in 0.1 N KOH solution.

Statistical analysis. Statistical significance was assessed within groups using the Student’s paired t-test and between groups by Dunnett’s multiple comparisons after one-way analysis of variance. Differences were considered statis-
tically significant if $P < 0.05$. All values are presented as means ± SE.

**RESULTS**

Effect of pharmacological interventions on HR and aortic pressure. Figure 1 shows typical recordings of the vagal stimulation frequency and the associated changes in HR in the absence and presence of isoproterenol infusion. HR changed in a manner roughly reciprocal to the stimulation pattern. Isoproterenol elevated the mean level of HR and augmented HR response to the dynamic vagal stimulation. As summarized in Table 1, all of the interventions studied increased the mean level of HR before and during dynamic vagal stimulation ($P < 0.05$). In the experiments with forskolin and with theophylline, we pretreated rabbits with propranolol (0.5 mg/kg iv). Therefore, the control HR levels of these groups tended to be lower than those of the isoproterenol group. These differences, however, did not reach a statistically significant level. Moreover, this dose of propranolol did not affect mean aortic pressure. Whereas neither isoproterenol nor theophylline significantly changed mean aortic pressure, forskolin decreased mean aortic pressure from $91 ± 4$ to $69 ± 6$ mmHg ($P < 0.05$).

Effect of isoproterenol on the transfer function. Figure 2A shows the transfer function from vagal stimulation frequency to HR response in the absence and presence of isoproterenol. The gain plots, phase plots, and coherences are shown. As already shown in our previous report (10), characteristics of the gain and phase plots match what is known as a first-order low-pass filter with time lag. The phase shift was nearly out-of-phase (i.e., $-\pi$ radians) at its lowest frequency and further delayed with increases in frequency. The coherence was above 0.8 in the frequency range from 0.01 to 0.3 Hz.

### Table 1. Effect of isoproterenol, forskolin, and theophylline on the mean level of HR and MAP before and during dynamic vagal stimulation

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>HR, beats/min</th>
<th>MAP, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treatment</td>
</tr>
<tr>
<td><strong>Isoproterenol (0.3 µg·kg$^{-1}$·min$^{-1}$ iv)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before stimulation</td>
<td>238 ± 9</td>
<td>305 ± 11†</td>
</tr>
<tr>
<td>During stimulation</td>
<td>191 ± 8</td>
<td>240 ± 8†</td>
</tr>
<tr>
<td><strong>Forskolin (5 µg·kg$^{-1}$·min$^{-1}$ iv)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before stimulation</td>
<td>227 ± 11</td>
<td>288 ± 13†</td>
</tr>
<tr>
<td>During stimulation</td>
<td>189 ± 9</td>
<td>211 ± 12*</td>
</tr>
<tr>
<td><strong>Theophylline (20 mg/kg iv)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before stimulation</td>
<td>211 ± 6</td>
<td>290 ± 8†</td>
</tr>
<tr>
<td>During stimulation</td>
<td>173 ± 4</td>
<td>202 ± 7†</td>
</tr>
</tbody>
</table>

Values are means ± SE. HR, heart rate; MAP, mean arterial pressure. *$P < 0.05$, †$P < 0.01$ vs. corresponding control values.
The high coherence values were similar to those reported previously (10, 11), indicating that the HR response linearly depends on the vagal stimulation frequency in this frequency range. Isoproterenol increased the gain of the transfer function. However, neither the phase shift nor the coherence was changed by the intervention. These observations are summarized in Table 2. Isoproterenol did not change the parameters of the transfer function, except for increasing the dynamic gain from $7.12 \pm 0.67$ to $12.4 \pm 1.21$ beats·min$^{-1}$·Hz$^{-1}$ ($P < 0.05$).

Figure 2B shows the calculated step responses in HR resulting from vagal stimulation in the absence and presence of isoproterenol. Isoproterenol augmented the maximum step response of HR to vagal stimulation at 1 Hz from $-7.03 \pm 0.75$ to $-14.0 \pm 1.22$ beats/min ($P < 0.05$). However, it did not change the dynamic response as characterized by the time constant of step response ($2.1 \pm 0.3$ vs. $2.1 \pm 0.2$ s).

Effect of forskolin on the transfer function. As shown in Fig. 3A, the effects of forskolin on the transfer function were similar to those of isoproterenol. Table 2 shows that forskolin did not change the parameters of the transfer function, except for increasing the dynamic gain from $7.20 \pm 1.15$ to $10.4 \pm 1.97$ beats·min$^{-1}$·Hz$^{-1}$ ($P < 0.05$).

Figure 3B demonstrates that forskolin increased the calculated step response. Forskolin increased the maximum response from $-6.96 \pm 1.02$ to $-11.4 \pm 1.58$ beats/min ($P < 0.05$) without changing the time constant ($1.9 \pm 0.3$ vs. $1.8 \pm 0.2$ s).

Effect of theophylline on the transfer function. As shown in Fig. 4A, theophylline increased the gain of the transfer function without altering coherence. Table 2 shows that theophylline increased the dynamic gain from $7.96 \pm 1.09$ to $12.5 \pm 1.27$ beats·min$^{-1}$·Hz$^{-1}$ ($P < 0.01$) and slightly decreased the corner frequency from $0.13 \pm 0.02$ to $0.11 \pm 0.02$ Hz ($P < 0.01$). However, theophylline did not change the lag time.

Figure 4B shows that theophylline increased the calculated maximum step response from $-7.73 \pm 0.94$ to $-13.9 \pm 1.27$ beats/min ($P < 0.01$). Theophylline tended to prolong the time constant ($1.7 \pm 0.2$ to $3.1 \pm 0.8$ s); however, the change did not reach significance.

**DISCUSSION**

We have shown that all pharmacological interventions to increase the level of intracellular cAMP examined in this study augmented the gain of the transfer function from vagal stimulation frequency to HR response. Isoproterenol and forskolin augmented cAMP synthesis via the indirect and direct activation, respectively, of adenylyl cyclase, whereas theophylline prevents cAMP hydrolysis by inhibiting PDE. The fact that the increase in gain of the transfer function was observed irrespective of the pharmacological mechanisms to increase cAMP suggested that it is nonspecifically coupled with the level of cAMP. Because the effects of these interventions on the transfer function were similar to those of simultaneous sympathetic stimulation in our previous study (10, 11), we conjecture that the accumulation of cAMP at postjunctional

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**Table 2. Effect of isoproterenol, forskolin, and theophylline on parameters of transfer function from vagal stimulation frequency to HR**

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>Dynamic Gain, beats·min$^{-1}$·Hz$^{-1}$</th>
<th>Corner Frequency, Hz</th>
<th>Lag Time, s</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treatment</td>
<td>Control</td>
</tr>
<tr>
<td>Isoproterenol (0.3 µg·kg$^{-1}$·min$^{-1}$ iv)</td>
<td>7.12 ± 0.67</td>
<td>12.4 ± 1.21$^*$</td>
<td>0.12 ± 0.03</td>
</tr>
<tr>
<td>Forskolin (5 µg·kg$^{-1}$·min$^{-1}$ iv)</td>
<td>7.20 ± 1.15</td>
<td>10.4 ± 1.97$^*$</td>
<td>0.12 ± 0.02</td>
</tr>
<tr>
<td>Theophylline (20 mg/kg iv)</td>
<td>7.96 ± 1.09</td>
<td>12.5 ± 1.27$^*$</td>
<td>0.13 ± 0.02$^*$</td>
</tr>
</tbody>
</table>

Values are means ± SE. *$P < 0.05$, †$P < 0.01$ vs. corresponding control values.
indicate mean transfer functions. Solid lines indicate means, and dashed lines indicate mean responses to 1-Hz tonic vagal stimulations shown for corresponding and dashed lines indicate mean 1

decomposition in corner frequency was not reflected in the time
decreased the corner frequency. Although the alteration before (n 7). Gains (top), phase shifts (middle), and coherence functions (bottom) are shown. Solid lines indicate means, and dashed lines indicate mean + SE values. B: calculated step responses to 1-Hz tonic vagal stimulations shown for corresponding transfer functions. Solid lines indicate means, and dashed lines indicate mean – SE values.

Fig. 4. A: Transfer functions from vagal stimulation frequency to HR change before (left) and after injections of theophylline (20 mg/kg iv; right) (pooled data; n = 7). Gains (top), phase shifts (middle), and coherence functions (bottom) are shown. Solid lines indicate means, and dashed lines indicate mean + SE values. B: Calculated step responses to 1-Hz tonic vagal stimulations shown for corresponding transfer functions. Solid lines indicate means, and dashed lines indicate mean – SE values.

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effector sites contributes, at least in part, to the sympathetic augmentation of the dynamic HR response to vagal stimulation.

In this study, we evaluated the influences of all pharmacological interventions on both the transfer function in the frequency domain and the calculated step response in the time domain. We derived the time domain representation of the dynamic characteristics of the transfer function in order to facilitate a somewhat intuitive interpretation of the system properties, even though the frequency domain representation faithfully provides the opportunity to understand the functional mechanisms of autonomic interaction involved in the control of HR. The gain and the corner frequency of the transfer function reflect the steady-state amplitude and time constant of the step response, respectively. Hence, the fact that all pharmacological interventions increased the gain of the transfer function means that the amplitude of the steady-state HR response to vagal stimulation was increased in all cases.Unlike isoproterenol and forskolin, theophylline alone slightly (~15%) decreased the corner frequency. Although the alteration in corner frequency was not reflected in the time constant of the calculated step response, the current data suggested that slowing of the degradation of cAMP might somewhat decelerate the HR response to vagal stimulation. Needless to say, however, this study indicated that the effect of theophylline was by far larger on the gain than on the corner frequency. Thus the augmented response of HR to vagal stimulation appears mainly attributable to accumulation of cAMP.

The mechanism by which the accumulated cAMP augments the dynamic HR response to vagal stimulation is not entirely clear. It is well known that the stimulation of muscarinic receptors directly alters the characteristics of the ACh-sensitive K⁺ channel (8), which, in turn, slows HR regardless of the level of cAMP. If intracellular cAMP accumulates, it is conceivable that the stimulation of muscarinic receptors may augment the negative chronotropic response by decreasing the level of cAMP through the inhibition of adenyl cyclase (4, 7, 19, 20) and through the acceleration of cAMP hydrolysis (5, 6, 12). Increases in mean HR resulting from the accumulation of cAMP might also affect the HR response to vagal stimulation. Additionally, a muscarinic antagonism of signal transduction with cAMP accumulation may also be operative (1, 15, 24).

Prejunctional interaction might also have been responsible for the augmented observation with concomitant sympathetic stimulation of the dynamic HR response to vagal stimulation. In prejunctional interaction, ACh released from vagus nerve terminals inhibits the release of NE from sympathetic nerve terminals, whereas NE and neuropeptide Y released from sympathetic nerve terminals inhibit the release of ACh from vagus nerve terminals (16). Our previous study has shown that simultaneous sympathetic stimulation did not affect the parameters of the transfer function from vagal stimulation frequency to HR response, except for increases in dynamic gain, despite the fact that vagal stimulation changed HR more quickly than did sympathetic stimulation (10). The data suggest that the reduction of NE release from sympathetic nerve terminals does not contribute substantially to the augmentation of the HR response to dynamic vagal stimulation. Moreover, because NE and neuropeptide Y inhibit the release of ACh from vagus nerve terminals (21, 24), if the inhibitory mechanism predominated, then the gain of the transfer function from vagal stimulation frequency to HR response would decrease. Therefore, it seems that prejunctional interaction may be less important in the sympathetic augmentation of the dynamic HR response to vagal stimulation.

Our previous report also suggested that the operating point of HR regulation would be determined through a sigmoidal relation between autonomic nervous activity and HR (10). This framework could explain bidirectional augmentation. Briefly, whenever we stimulated the sympathetic system or the vagal system alone, the operating point of HR deviated from the steepest region of the sigmoidal curve, resulting in a loss of gain in the HR response. Simultaneous stimulation of the
sympathetic and vagal systems moved the operating point back to the steepest region of the sigmoidal curve, thereby increasing the HR response to dynamic stimulation. In this study, we selected doses of each drug so as to mimic the HR response to sympathetic stimulation observed in our previous studies (10); therefore, these interventions should also shift the operating point and increase the gains of the transfer functions. However, the framework predicts that extremely low or high levels of sympathetic stimulation would result in a decrease in the dynamic HR response to vagal stimulation. Hence, the smaller and the larger doses of each drug used in this study may fail to increase the gain of transfer function of vagal stimulation frequency to HR response. Indeed, the presence of an effective stimulation range necessary for eliciting observable interactions between the sympathetic and the vagal system in regulating cardiac function have been shown previously (7, 23). Obviously, further studies are required to validate this framework.

In summary, we found that pharmacological interventions, which mimicked the sympathetic-mediated cardiac response, increased the gain of the transfer function from vagal stimulation frequency to HR response. These results suggest that the accumulation of cAMP at postjunctional effector sites contributes, at least in part, to the sympathetic augmentation of the dynamic HR response to vagal stimulation. Because the data presented here were obtained from experiments using anesthetized rabbits, the relative significance of cAMP under normal physiological conditions must be evaluated in careful investigations using conscious rabbits. Furthermore, to understand further the mechanisms of bidirectional augmentation, we should elucidate whether the vagal augmentation of HR response to dynamic sympathetic stimulation could be mediated through the adenylyl cyclase system. Nevertheless, we would like to stress here that this is the first report that the mechanism of muscarinic inhibition of the cardiac Ca current. Pflügers Arch. 407: 182–189, 1986.


