Neuronal uptake affects dynamic characteristics of heart rate response to sympathetic stimulation

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Nakahara, Tsutomu, Toru Kawada, Masaru Sugimachi, Hiroshi Miyano, Takayuki Sato, Toshiaki Shishido, Ryoichi Yoshimura, Hiroshi Miyashita, Masashi Inagaki, Joe Alexander, Jr., and Kenji Sunagawa. Neuronal uptake affects dynamic characteristics of heart rate response to sympathetic stimulation. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R140–R146, 1999.—Recently, studies in our laboratory involving the use of a Gaussian white noise technique demonstrated that the transfer function from sympathetic stimulation frequency to heart rate (HR) response showed dynamic characteristics of a second-order low-pass filter. However, determinants for the characteristics remain to be established. We examined the effect of an increase in mean sympathetic stimulation frequency and that of a blockade of the neuronal uptake mechanism on the transfer function in anesthetized rabbits. We found that increasing mean sympathetic stimulation frequency from 1 to 4 Hz significantly (P < 0.01) decreased the dynamic gain of the transfer function without affecting other parameters, such as the natural frequency, lag time, or damping coefficient. In contrast, the administration of desipramine (0.3 mg/kg iv), a neuronal uptake blocking agent, significantly (P < 0.01) decreased both the dynamic gain and the natural frequency and prolonged the lag time. These results suggest that the removal rate of norepinephrine at the neuroeffector junction, rather than the amount of available norepinephrine, plays an important role in determining the low-pass filter characteristics of the HR response to sympathetic stimulation.

Dynamic stimulation; Gaussian white noise; neuronal uptake mechanism; systems analysis; transfer function

SYMPATHETIC STIMULATION leads to cardiac responses by releasing norepinephrine (NE) from the sympathetic nerve terminals. The released NE binds to β-adrenoceptors and triggers a cascade of events, including activation of the stimulatory G protein, stimulation of adenylyl cyclase, synthesis of cAMP, activation of cAMP-dependent protein kinases, and phosphorylation of various substrates, such as ion channels (6, 7). On the other hand, most of the released NE is removed by a neuronal uptake mechanism (3). A recent study from our laboratory using dynamic systems analysis has shown that the transfer function from dynamic sympathetic stimulation frequency to heart rate (HR) response well approximates the characteristics of a second-order low-pass filter (8). However, determinants of the filter characteristics remain to be elucidated.

In the previous paper (8), we parameterized the transfer function from sympathetic stimulation frequency to HR response by steady-state gain, natural frequency, damping coefficient, and lag time. Conceptually, all of the steps involved in the signal transduction sequence from sympathetic stimulation to HR can affect the gain of the transfer function, because the total gain is a product of all the gains relating respective steps. By contrast, a rate-limiting step, which determines how quickly the system can respond to dynamic changes in the input, mainly affects the parameters of the low-pass filter, such as the natural frequency and the damping coefficient.

It is well documented that blockade of the neuronal uptake mechanism markedly affects the time course of the chronotropic responses to sympathetic stimulation (11, 13–15). Therefore, NE kinetics at the neuroeffector junction of the sinus node, rather than post-junctional intracellular signaling processes, might be important in determining the dynamic characteristics of HR response to sympathetic stimulation. Thus the purpose of this study was to examine how changes in NE kinetics at the neuroeffector junction affect the dynamic gain and/or low-pass filter characteristics of HR response to sympathetic stimulation. We altered NE kinetics by both changing mean sympathetic stimulation frequency and administrating a neuronal uptake blocker. To estimate the transfer function from sympathetic stimulation frequency to HR response, we stimulated the right cardiac sympathetic nerve according to a Gaussian white noise pattern in anesthetized rabbits.

MATERIALS AND METHODS

Surgical preparations. Animal care was in accordance with the guidelines of the Physiological Society of Japan. Fourteen Japanese white rabbits weighing 2.4–3.0 kg were anesthetized with doses of urethan (250 mg/kg iv) and α-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, 3F; Millar Instrument, Houston, TX) inserted via the left femoral artery. Another catheter was inserted into the right femoral vein for the administration of drugs. The

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cardiovascular responses to baroreflex stimulation, we performed experiments with constant sympathetic nerve stimulation. The pulse duration of nerve stimulation was set at 2 ms. We adjusted the amplitude of sympathetic nerve stimulation to yield a HR increase of about 50 beats/min at a 2-Hz constant stimulation. This resulted in an optimal stimulation level to avoid confusing mean stimulation frequency of the sympathetic nerve with the modulation frequency of dynamic sympathetic stimulation.

In the second series of experiments (n = 7 animals), we examined the effects of desipramine (Sigma, St. Louis, MO) on the transfer function from sympathetic nerve stimulation frequency to HR response. We recorded the HR response to the dynamic sympathetic stimulation with Gaussian white noise (1 ± 0.5 Hz) under control conditions. We then repeated the same stimulation protocol in the presence of desipramine (0.03 and 0.3 mg/kg iv). The dynamic sympathetic stimulation was started 15–20 min after bolus injection of each dose. After reaching steady state in each stimulation protocol, we recorded both the stimulation frequency and HR for 10 min.

Heart rate and command sequences were digitized at 200 Hz with 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 HR before sympathetic nerve stimulation by averaging instantaneous HR for 10 s before the stimulation. The mean HR during sympathetic nerve stimulation was calculated by averaging instantaneous HR for a given time period (10 min).

Estimation of the transfer function. We resampled the input-output (stimulation frequency-HR) data pairs at 10 Hz by averaging 20 successive points, 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 the HR data. The frequency resolution calculated as a reciprocal of the segment length (102.4 s) was 0.00977 Hz.

We ensemble-averaged the power of the nerve stimulation [SN·N(f)], the HR response [SHR·HR(f)], and the cross power between them [SN·HR(f)] over the eight segments. Finally, we obtained the transfer function [H(f)], relating nerve stimulation frequency to HR response with the following equation

\[ H(f) = \frac{SN·HR(f)}{SN·N(f)} \]

The modulus [|H(f)|] and phase shift [\[\angle H(f)\]] of the transfer function were derived from the real [\Re{H(f)}] and imaginary [\Im{H(f)}] parts of the complex transfer function.
parts \[|H(\omega)| = \sqrt{H_r(\omega)^2 + H_i(\omega)^2}\]

\[\theta(\omega) = \tan^{-1}\frac{H_i(\omega)}{H_r(\omega)}\]

The modulus indicates the amplitude of HR change per unit change of nerve stimulation frequency and is expressed in beats per minute per Hertz. 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 at each frequency.

Because the transfer function from sympathetic stimulation frequency to HR response approximated a second-order low-pass filter with lag time (8), we parameterized the transfer function by using the following equations

\[H(\omega) = \frac{K}{1 + 2\zeta \frac{\omega}{\omega_n} - (\frac{\omega}{\omega_n})^2 e^{-2\zeta\omega L}}\]

where \(\omega\) is the frequency, and \(K, \zeta, \omega_n,\) and \(L\) are parameters characterizing the system. \(K\) is a steady-state gain of the system. The parameter \(\zeta\) is a damping coefficient. Depending on the value of the damping coefficient, the system behaves as underdamped \((0 < \zeta < 1)\), as critically damped \((\zeta = 1)\), or as overdamped \((\zeta > 1)\). The parameter \(\omega_n\) is a natural frequency and affects the upper frequency bandwidth of the system response. \(L\) is a lag time, which is defined as a delay between the input and the output signals without any distortion of the waveform (16). We calculated these parameters using an iterative, nonlinear, least-squares fitting technique (2).

To quantify the linear dependence of the HR response on nerve stimulation, we estimated the coherence function \([\text{Coh}(\omega)]\) through use of the following equation

\[\text{Coh}(\omega) = \frac{|S_n \cdot HR(\omega)|^2}{S_n \cdot N(\omega) \cdot S_H \cdot R(\omega)}\]

The coherence value ranges between 0 and unity. A unity coherence indicates a perfect linear dependence between the input and output, whereas zero coherence indicates total independence between the two signals.

Statistical analysis. Statistical significance was assessed by Dunnett’s multiple comparison performed after one-way ANOVA. Differences were considered statistically significant if \(P < 0.05\). All values are presented as means ± SD.

RESULTS

Effect of stimulation frequency level on transfer function. Figure 1 shows typical recordings of changes in HR in response to dynamic sympathetic stimulation with frequency-modulated Gaussian white noise. HR changed in a fashion that roughly paralleled the stimulation pattern. Increases in sympathetic stimulation level elevated the mean HR during dynamic stimulation. The HR response to dynamic sympathetic stimulation, however, appeared to be attenuated at the stimulation level of 4 Hz.

Table 1. Effect of changes in frequencies of sympathetic stimulation on mean heart rate

<table>
<thead>
<tr>
<th>Frequencies of Dynamic Sympathetic Stimulation, Hz</th>
<th>Heart rate, beats/min</th>
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<tbody>
<tr>
<td>1 ± 0.5</td>
<td>234 ± 11</td>
</tr>
<tr>
<td>2 ± 1</td>
<td>234 ± 14</td>
</tr>
<tr>
<td>4 ± 2</td>
<td>240 ± 14</td>
</tr>
</tbody>
</table>

Values are means ± SD. *P < 0.01 vs. corresponding 1 ± 0.5 Hz values. †P < 0.01 vs. corresponding prestimulation values.

Fig. 2. Transfer functions from sympathetic stimulation frequency to HR response obtained at stimulation frequencies of 1 ± 0.5 (left), 2 ± 1 (middle), and 4 ± 2 Hz (right) (pooled data; \(n = 7\) animals). Gains (Gain), phase shifts (Phase) and coherence functions (Coh) are shown. Note that gain and frequency axes are logarithmically scaled. Solid lines indicate means; broken lines indicate means ± SD. rad, Radians.
As summarized in Table 1, sympathetic stimulation increased the mean HR relative to prestimulation values (P < 0.01) at stimulation levels of 1, 2, and 4 Hz. The mean HR during the stimulation level of 1 Hz was lower than mean HRs during stimulation levels of 2 and 4 Hz (P < 0.01). However, there was no sizable difference in mean HRs between the stimulation levels of 2 and 4 Hz. Thus the HR response appeared to be saturated at the stimulation level of 4 Hz.

Figure 2 shows the transfer functions obtained at sympathetic stimulation levels of 1, 2, and 4 Hz. The gain plots, phase plots, and coherence functions are shown. Characteristics observed in the gain and phase plots match what is known as a second-order low-pass filter with lag time. The phase plots approached zero radians at their lowest frequencies and delayed as frequency increased. For all three stimulation levels, the coherence was above 0.8 in the frequency range from 0.01 to 0.1 Hz. The dynamic gain decreased as the sympathetic stimulation level was increased. In contrast, neither the phase shifts of the transfer functions nor the coherence functions differed remarkably among the three conditions.

Table 2. Effect of desipramine on mean heart rate

<table>
<thead>
<tr>
<th>Heart rate, beats/min</th>
<th>Desipramine mg/kg iv</th>
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<tbody>
<tr>
<td><strong>Control</strong></td>
<td></td>
</tr>
<tr>
<td>During prestimulation</td>
<td></td>
</tr>
<tr>
<td>226 ± 17</td>
<td>233 ± 14</td>
</tr>
<tr>
<td>During dynamic sympathetic stimulation</td>
<td>258 ± 14*</td>
</tr>
<tr>
<td>260 ± 16†</td>
<td>269 ± 13†</td>
</tr>
<tr>
<td>273 ± 15†</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD. *P < 0.01 vs. corresponding control values. †P < 0.01 vs. corresponding prestimulation values.

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Shown in Fig. 3 are summary results of the parameters characterizing the transfer functions presented in Fig. 2. Errors introduced by the fitting procedure were 11.4 ± 10.9% of the gain and 0.15 ± 0.13 radians of the phase in the frequency range 0.01 to 0.1 Hz. As can be seen, increasing the sympathetic stimulation level significantly (P < 0.01) decreased the dynamic gain of the transfer function, whereas neither the natural frequency, the damping coefficient, nor the lag time varied with changes in the sympathetic stimulation level.

Effect of desipramine on transfer function. Figure 4 shows typical recordings of dynamic sympathetic stimulation frequency and associated HR responses obtained before and after desipramine (0.03 and 0.3 mg/kg iv). Desipramine increased mean HR while attenuating the dynamic HR response to sympathetic stimulation in a dose-dependent manner.

As summarized in Table 2, intravenous desipramine (0.3 mg/kg) significantly (P < 0.01) increased mean HR
before sympathetic stimulation. Sympathetic stimulation significantly \((P < 0.01)\) increased HR both in the absence and presence of desipramine. Although desipramine showed a tendency to increase mean HR during sympathetic stimulation, the changes did not reach statistical significance.

Figure 5 shows the effects of desipramine on the transfer function from dynamic sympathetic stimulation frequency to HR response. The gain plots, phase plots, and coherence functions are shown. Desipramine decreased the dynamic gain in a dose-dependent manner. It also increased the phase shift at any given frequency. The coherence was above 0.8 in the frequency range from 0.01 to 0.1 Hz before desipramine. However, increasing the dose of desipramine lowered the coherence to some extent.

Illustrated in Fig. 6 are the parameters characterizing the transfer function presented in Fig. 5. Desipramine decreased the dynamic gain in a dose-dependent manner. In contrast to increasing the sympathetic stimulation level, desipramine lowered the natural frequency and prolonged the lag time. However, desipramine did not affect the damping coefficient.

DISCUSSION

We have demonstrated that increases in the sympathetic stimulation level decrease the gain of the transfer function from sympathetic stimulation frequency to HR response without changing the parameters of low-pass filter characteristics. On the contrary, desipramine not only decreased the gain but also affected the natural frequency and lag time of the transfer function. These results suggest that the NE uptake process at the neuroeffector junction, rather than the amount of available NE, determines the low-pass filter characteristics of HR response to sympathetic stimulation.

Changes in dynamic gain of the transfer function. Both increases in sympathetic stimulation level (Figs. 2 and 3) and administration of desipramine (Figs. 5 and 6) decrease the dynamic gain of the transfer function. Previous investigations have indicated that the effect of
sympathetic stimulation on HR depends on a system operating point (i.e., the mean HR during sympathetic stimulation) (1, 8). Namely, increasing sympathetic stimulation levels shifts the operating point of HR to a saturating zone of the sympathetic effect on HR, thereby decreasing the dynamic gain of the transfer function. Our observation of a decreased dynamic gain with increased sympathetic stimulation level follows this framework. In contrast, desipramine did not markedly increase the mean HR during sympathetic stimulation while decreasing the dynamic gain. Therefore, changes in the mean HR during sympathetic stimulation alone cannot account for the decreased dynamic gain after the administration of desipramine.

Increased NE concentration at the neuroeffector junction activates the presynaptic \( \alpha \)-adrenoceptors, which attenuate the effect of sympathetic stimulation on HR (4, 10, 18, 21). Although this mechanism is common for both the stimulation- and desipramine-induced NE elevation, the relative significance of the presynaptic inhibitory regulation might be different between the two conditions. The prestimulation increase in the mean HR induced by desipramine might also cause the desensitization of intracellular signal transduction pathways mediated by sympathetic nerves, thereby decreasing the dynamic gain.

Changes in low-pass filter characteristics of the transfer function. Unlike increased sympathetic stimulation levels, desipramine affected not only the dynamic gain, but also the natural frequency and lag time of the transfer functions. Previous reports have shown that blocking of the neuronal uptake mechanism decelerates the off-response much more severely than the on-response of HR to sympathetic stimulation (11, 13–15). The on-response indicates the HR response to the onset of sympathetic stimulation, whereas the off-response indicates the HR response to the termination of sympathetic stimulation. Because of its fundamental assumption of linearity, the transfer function analysis does not permit a distinction between the on- and off-responses of HR to sympathetic stimulation. However, a decrease in the natural frequency by desipramine is most likely a manifestation of the decelerated off-response of HR to sympathetic stimulation. By contrast, the reasons for lag time prolongation are presently unknown. Because desipramine does not affect intracellular processes coupled to the \( \beta \)-adrenoceptor (11, 14), the prolongation of the lag time by desipramine may be attributable to prejunctional processes.

The fact that the modulation of the prejunctional processes by desipramine affects the low-pass filter characteristics of the transfer function suggests that the rate of postjunctional processes is faster than or similar to that of prejunctional processes. In regard to postjunctional processes, studies on cardiac myocytes suggest that adenylyl cyclase activation, cAMP accumulation, and cAMP-dependent processes are the rate-limiting steps in the \( \beta \)-adrenergic response (i.e., activation of L-type \( \text{Ca}^{2+} \) inward currents and of \( \text{Cl}^- \) currents) (5, 17, 20). However, in these in vitro experiments, even when isoproterenol was quickly applied to isolated myocytes, a marked latency (2–5 s) was observed before the activation of these currents. The latency was even longer than our estimated lag time (~1 s) from sympathetic stimulation to HR response. Because the lag time of one part of the system should be shorter than that of the total system, it is difficult to extrapolate the findings of in vitro experiments into interpretations of the in vivo HR response.

In summary, we demonstrated that desipramine markedly changes the parameters of low-pass filter characteristics of HR response to sympathetic stimulation. Thus the low-pass filter characteristics of the HR response to sympathetic stimulation may be primarily attributable to NE kinetics at the neuroeffector junction. Among the multiple processes that are involved in NE kinetics at the neuroeffector junction, we concluded that the removal of NE through the neuronal uptake mechanism plays an important role in determining the low-pass filter characteristics of HR response to sympathetic stimulation.

Perspectives

These results reinforce the importance of NE kinetics at the neuroeffector junction of the sinus node in determining the dynamic sympathetic regulation of HR. Because the transfer function from sympathetic stimulation frequency to HR response reflects changes in NE kinetics at the neuroeffector junction in the sinus node, simultaneous recordings of cardiac sympathetic nerve activity and HR would allow us to evaluate NE kinetics in in vivo experiments. If the dynamic gain decreases without changing the low-pass filter characteristics from sympathetic nerve activity to HR, we may exclude the insufficiency of a neuronal uptake mechanism.

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REFERENCES