Effects of neuronal norepinephrine uptake blockade on baroreflex neural and peripheral arc transfer characteristics

Toru Kawada,1 Tadayoshi Miyamoto,1,2 Kazunori Uemura,1 Koji Kashihara,1,3 Atsunori Kamiya,1 Masaru Sugimachi,1 and Kenji Sunagawa1
1Department of Cardiovascular Dynamics, National Cardiovascular Center Research Institute, Osaka 565–8565; and 2Japan Association for the Advancement of Medical Equipment, Tokyo 105–0013; and 3Organization for Pharmaceutical Safety and Research, Tokyo 100–0013, Japan

Cardiovascular Dynamics, National Cardiovascular Center Research Institute, Fujishirodai, Suita, Osaka 565–8565, Japan (E-mail: torukawa@res.ncve.go.jp).

Effects of neuronal norepinephrine uptake blockade on baroreflex neural and peripheral arc transfer characteristics. Am J Physiol Regul Integr Comp Physiol 286: R1110–R1120, 2004. First published February 12, 2004; 10.1152/ajpregu.00527.2003.—Neuronal uptake is the most important mechanism by which norepinephrine (NE) is removed from the synaptic clefts at sympathetic nerve terminals. We examined the effects of neuronal NE uptake blockade on the dynamic sympathetic regulation of the arterial baroreflex because dynamic characteristics are important for understanding the system behavior in response to exogenous disturbance. We perturbed intracarotid sinus pressure (CSP) according to a binary white noise sequence in anesthetized rabbits, while recording cardiac sympathetic nerve activity (SNA), arterial pressure (AP), and heart rate (HR). Intravenous administration of desipramine (1 mg/kg) decreased the normalized gain of the neural arc transfer function from CSP to SNA relative to untreated control (1.03 ± 0.09 vs. 0.60 ± 0.08 AU/mmHg, mean ± SE, P < 0.01) but did not affect that of the peripheral arc transfer function from SNA to AP (1.10 ± 0.05 vs. 1.08 ± 0.10 mmHg/AU). The normalized gain of the transfer function from SNA to HR was unaffected (1.01 ± 0.04 vs. 1.09 ± 0.12 beats·min−1·AU−1). Desipramine decreased the natural frequency of the transfer function from SNA to AP by 28.7 ± 7.0% (0.046 ± 0.007 vs. 0.031 ± 0.002 Hz, P < 0.05) and that of the transfer function from SNA to HR by 64.4 ± 2.2% (0.071 ± 0.003 vs. 0.025 ± 0.002 Hz, P < 0.01). In conclusion, neuronal NE uptake blockade by intravenous desipramine administration reduced the total buffering capacity of the arterial baroreflex mainly through its action on the neural arc. The differential effects of neuronal NE uptake blockade on the dynamic AP and HR responses to SNA may provide clues for understanding the complex pathophysiology of cardiovascular diseases associated with neuronal NE uptake deficiency.

Address for reprint requests and other correspondence: T. Kawada, Dept. of Cardiovascular Dynamics, National Cardiovascular Center Research Institute, 5–7–1 Fujishirodai, Suita, Osaka 565–8565, Japan (E-mail: torukawa@res.ncve.go.jp).

NOREPINEPHRINE (NE) is released from sympathetic nerve terminals in proportion to nerve activity and removed primarily by a neuronal uptake mechanism (27). Deficiency of neuronal NE uptake results in accumulation of NE in the synaptic cleft, leading to malfunction of the dynamic sympathetic regulation of target organs. For instance, Nakahara et al. (26) demonstrated that intravenous administration of the neuronal NE uptake blocker desipramine decelerated the dynamic heart rate (HR) response to electrical stimulation of the cardiac sympathetic nerve. When the transfer function from sympathetic nerve stimulation to HR was parameterized using a second-order low-pass filter, desipramine decreased dynamic gain and lowered natural frequency of the low-pass filter. On the other hand, Bertram et al. (5) demonstrated that desipramine decreased dynamic gain but did not markedly alter natural frequency of the transfer function from lumbar sympathetic nerve stimulation to the hindlimb vascular conductance response. Although the effects of neuronal NE uptake blockade on the dynamic sympathetic regulation have been examined using electrical stimulation of the sympathetic nerves, electrical sympathetic nerve stimulation can differ from native sympathetic discharge in its effects on target organs due to the differences in discharge pattern (9). Accordingly, the effects of neuronal NE uptake blockade on the dynamic sympathetic regulation should be reexamined using native sympathetic discharge. Further, the effects of neuronal NE uptake blockade on the overall open-loop baroreflex transfer function remain to be elucidated. Because deficiency of neuronal NE uptake is associated with a subgroup of patients with orthostatic intolerance (32, 33) or essential hypertension (30), elucidating the effects of neuronal NE uptake blockade on dynamic baroreflex function would contribute to better understanding the pathology underlying such cardiovascular diseases.

Dorward et al. (11) compared the effects of central and peripheral desipramine administrations on sympathoadrenal function and HR. In their study, intracisternal administration of desipramine attenuated changes in renal sympathetic nerve activity (SNA) in response to slow ramp changes in mean arterial pressure (AP), whereas it minimally affected the HR response. In contrast, intravenous administration of desipramine caused a baroreceptor-independent reduction in renal SNA and an augmentation of the HR response to slow ramp changes in mean AP. Although these results suggest that desipramine modulates the baroreflex function through both central and peripheral actions, how the intravenous desipramine modulates dynamic baroreflex characteristics remains to be elucidated. Bertram et al. (5) demonstrated that intravenous desipramine attenuated the dynamic AP response to the aortic depressor nerve stimulation. However, whether their results were caused by attenuation of the SNA response to depressor nerve stimulation or by attenuation of the AP response to SNA was unanswered.

We hypothesized that neuronal NE uptake blockade would blunt the dynamic sympathetic regulations of AP and HR. We
performed an open-loop experiment on the carotid sinus baroreflex in anesthetized rabbits, wherein systemic SNA was altered by way of the baroreflex (16, 17, 19–22). This experimental design enabled us to separately assess the baroreflex neural and peripheral arc transfer characteristics. The baroreflex neural arc refers to the input-output relationship between baroreceptor input pressure and SNA, whereas the baroreflex peripheral arc refers to that between SNA and AP. The following conclusions were reached from the present investigation: 1) the neuronal NE uptake blockade by intravenous desipramine administration decreased dynamic gain of the total baroreflex mainly through attenuation of the neural arc gain, and 2) the effects of neuronal NE uptake blockade were found to be stronger in the dynamic HR response than in the dynamic AP response to native sympathetic discharge.

MATERIALS AND METHODS

Surgical preparations. All animals used in this study were cared for in strict accordance with Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences, approved by the Physiological Society of Japan. Fourteen Japanese White rabbits weighing 2.7–3.6 kg were anesthetized via intravenous injection (2 ml/kg) of a mixture of urethane (250 mg/ml) and α-chloralose (40 mg/ml) and were mechanically ventilated with oxygen-enriched room air. Supplemental doses of these anesthetics were intravenously administered (0.2–0.3 ml-kg⁻¹-h⁻¹) to maintain the appropriate level of anesthesia. AP was measured using a high-fidelity pressure transducer (Millar Instruments, Houston, TX) inserted into the right femoral artery. AP was monitored using a high-fidelity pressure transducer (Millar Instruments, Houston, TX) inserted into the right femoral artery.

After completing the surgical preparation, the baroreflex neural arc transfer function from CSP to SNA (H\textsubscript{CSP-SNA}), peripheral arc transfer function from SNA to AP (H\textsubscript{SNA-AP}), and total baroreflex loop transfer function from CSP to AP (H\textsubscript{CSP-AP}) were estimated. The baroreflex function estimation was performed based on a discrete proportional-integrative-derivative control algorithm (2). The servocontroller used for this experiment was composed of a microcomputer and a commercial device that was designed to reproduce the changes in HR according to the baroreflex function. The servocontroller was set to operate based on a discrete proportional-integrative-derivative control algorithm (2). The servocontroller used for this experiment was composed of a microcomputer and a commercial device that was designed to reproduce the changes in HR according to the baroreflex function. The servocontroller was set to operate at a rate of 200 Hz using a 12-bit analog-to-digital converter. We calculated the system step response as follows. We obtained the ensemble averaged autospectral densities of the input [S\textsubscript{XX}(f)] and output [S\textsubscript{YY}(f)], and cross-spectral density between the input and output [S\textsubscript{XY}(f)] over the six segments. Finally, we calculated the gain function from the division of S\textsubscript{XY}(f) by S\textsubscript{XX}(f) (3, 25, 29, 34) (see Eq. A4 in APPENDIX).

To quantify the linear dependence between the input and output signals in the frequency domain, we calculated a magnitude-squared coherence function \(\text{Coh}(f)\) using the following equation (3, 25, 29):

\[
\text{Coh}(f) = \frac{|S_{XY}(f)|^2}{S_{XX}(f)S_{YY}(f)}
\]

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

To facilitate the interpretation of the estimated transfer function, we calculated the system step response as follows. We obtained the impulse response from the real part of the inverse Fourier transformation of the transfer function and then calculated the step response from the time integral of the impulse response.

Statistical analysis. All data are expressed as means ± SE. In protocol 1, SNA was normalized in each animal so that the background noise level averaged for 1 min was zero and the SNA value averaged for the first minute in the control (DMI\textsubscript{0}) condition was 100 arbitrary units (AU). We calculated mean SNA, AP, and HR by averaging respective instantaneous values for 10 min during binary white noise perturbation under DMI\textsubscript{0}, DMI\textsubscript{0.3}, and DMI\textsubscript{1.0} conditions.

To compare transfer functions in protocol 1, we determined a normalization factor so that a transfer gain value averaged below 0.024 Hz became unity in the DMI\textsubscript{0} condition. The same normalization factor was then applied to the transfer functions obtained from the DMI\textsubscript{0.3} and DMI\textsubscript{1.0} conditions. In protocol 2, we normalized transfer functions so that the transfer gain value averaged below 0.024 Hz became unity in the Ctl1 condition.
We parameterized $H_{\text{CSP-SNA}}$ using the following model according to previous studies (21, 22)

$$H_s(f) = \frac{K}{1 + \frac{\zeta f_s}{f_c} f^2 + \left(\frac{f}{f_s}\right)^2} \exp(-2\pi f j L)$$ (2)

where $K, f_c, f_s, \zeta$, and $L$ represent steady-state gain, corner frequency relating to derivative characteristics (in Hz), corner frequency relating to high-cut characteristics (in Hz), and pure delay (in s), respectively, and $j$ represents imaginary units. The negative sign of the right-hand side of Eq. 2 indicates the negative feedback attained by the baroreflex neural arc.

We parameterized $H_{\text{SNA-AP}}$ and $H_{\text{SNA-HR}}$ using a second-order low-pass filter with pure delay according to previous studies as follows (5, 16, 18, 22, 26)

$$H_s(f) = \frac{K}{1 + \frac{\zeta f_s}{f_c} f^2 + \left(\frac{f}{f_s}\right)^2} \exp(-2\pi f j L)$$ (3)

where $K, f_c, f_s, \zeta$, and $L$ represent steady-state gain, natural frequency (in Hz), damping ratio, and pure delay (in s), respectively.

With respect to $H_{\text{CSP-AP}}$, although the product of Eqs. 2 and 3 might yield a model transfer function for the total baroreflex loop, we did not adopt such a model because an excess number of parameters leads to large variance in the parameter estimation. Instead, we used a first-order low-pass filter with pure delay to parameterize $H_{\text{CSP-AP}}$ as follows (16)

$$H_s(f) = \frac{K}{1 + \frac{\zeta f_s}{f_c} f^2} \exp(-2\pi f j L)$$ (4)

where $K, f_c$, and $L$ represent steady-state gain, corner frequency (in Hz), and pure delay (in s), respectively. Some reasoning for using Eq. 4 rather than the product of Eqs. 2 and 3 is discussed below. First, $f_s$ and $f_c$ in Eq. 2 can be omitted, because the transfer gain of $H_{\text{CSP-AP}}$ decreases significantly at the frequency of $f_c$ ($\approx 0.8$ Hz in rabbits) and the inclusion of $f_s$ does not improve the fitting accuracy for $H_{\text{CSP-AP}}$ much. Second, because $f_c$ in Eq. 2 is close to $f_s$ in Eq. 3, zero-pole cancellation occurs in the product of Eqs. 2 and 3, leaving one pole ($f_c$) in Eq. 4.

To quantify the goodness of fit of the transfer function, we calculated the following value

$$q = 1 - \sum_{i=1}^{M} \left(\frac{\left|\mathcal{H}(f_i) - G(f_i)\right|^2}{\sum_{i=1}^{M} \left|\mathcal{H}(f_i)\right|^2}\right) \times f_i = f_0 \times i$$ (5)

where $\mathcal{H}(f)$ and $G(f)$ indicate measured and model transfer functions, respectively, and $f_0$ indicates the fundamental frequency of Fourier transformation (0.0078 Hz). When $G(f)$ exactly matches $\mathcal{H}(f)$, $q$ becomes unity; $q$ decreases from unity as the deviation of $G(f)$ from $\mathcal{H}(f)$ increases. For $H_{\text{CSP-SNA}}$, we fit the transfer function up to 1 Hz ($M = 128$). For $H_{\text{SNA-AP}}, H_{\text{SNA-HR}},$ and $H_{\text{CSP-AP}}$, we fit the transfer function up to 0.5 Hz ($M = 64$) because the transfer function estimation became less reliable in the higher frequencies due to decreased output amplitude associated with the low-pass characteristics.

To examine the differences in the step response among DMI0, DMI0.3, and DMI1.0 conditions in protocol 1, we calculated the steady-state step response by averaging the step response between 50 and 60 s. For the step response relating to $H_{\text{CSP-SNA}}$, the peak negative response and the time to peak were calculated. For the step responses relating to $H_{\text{SNA-AP}}, H_{\text{SNA-HR}},$ and $H_{\text{CSP-AP}}$, the rise time was calculated as the time required for the step response to traverse the region between 10% and 80% of the steady-state response.

In protocol 1, the effects of neuronal NE uptake blockade on the respective transfer functions were examined using Dunnett’s multiple comparison test following repeated-measures ANOVA (14). Mean SNA, AP, and HR were calculated among DMI0, DMI0.3, and DMI1.0 conditions using the same statistical procedure. Goodness of fit and coherence values were compared against DMI0 using nonparametric multiple comparison based on ranks following Friedman test (14) as the normal distribution may not be applicable to these quantities. To examine the differences in the AP and HR responses to SNA, the parameters attained by fitting Eq. 3 to $H_{\text{SNA-AP}}$ and $H_{\text{SNA-HR}}$ under DMI0 condition were compared by paired t-test.

In protocol 2, differences in the transfer function parameters between Ct1 and Ct2 conditions were examined using paired t-test. Differences in goodness of fit and coherence values between Ct1 and Ct2 conditions were examined using Wilcoxon signed-rank test (14) as the normal distribution may not be applicable to these quantities.

RESULTS

Figure 1 shows typical time series of CSP, SNA, AP, and HR obtained under DMI0, DMI0.3, and DMI1.0 conditions in protocol 1. CSP was perturbed according to a binary white noise sequence. SNA, AP, and HR were changed dynamically in response to the CSP perturbation. Intravenous administration of desipramine decreased mean SNA, resulting in the decreased SNA variation in both DMI0.3 and DMI1.0 compared with DMI0. Although mean AP decreased in DMI1.0 compared with DMI0 in Fig. 1, changes in mean AP were statistically insignificant across the animals (Table 1). Mean HR was elevated in both DMI0.3 and DMI1.0 compared with DMI0.

Mean CSP, SNA, AP, and HR in protocol 1 are summarized in Table 1. Mean CSP was unchanged among the three conditions. Mean SNA was lower in both DMI0.3 and DMI1.0 than in DMI0. Mean AP did not change among the three conditions. Mean HR was higher in both DMI0.3 and DMI1.0 than in DMI0.

Figure 2A shows the group-averaged $H_{\text{CSP-SNA}}$ obtained from protocol 1. Gain plots, phase plots, and coherence functions are presented. The transfer gain increased as the frequency increased from 0.05 to 0.5 Hz in each condition, indicating derivative characteristics of the baroreflex neural arc. Intravenous desipramine administration shifted the gain plot downward in a frequency-independent manner. The phase approached $-\pi$ radians at the lowest frequency in each condition, reflecting the out-of-phase relationship between CSP and SNA at the steady state. The coherence value was below 0.5 at 0.01 Hz and increased to $\sim 0.7$ between 0.1 and 0.4 Hz in DMI0. The coherence values averaged up to 1.0 Hz were 0.68 $\pm$ 0.03, 0.55 $\pm$ 0.03, and 0.52 $\pm$ 0.04 under the DMI0, DMI0.3, and DMI1.0 conditions, respectively. The averaged coherence was significantly smaller in DMI1.0 than in DMI0 ($P < 0.05$).

Figure 2B shows the SNA step response derived from $H_{\text{CSP-SNA}}$. Desipramine significantly attenuated the negative peak response from $-3.60 \pm 0.40$ to $-2.42 \pm 0.40$ ($P < 0.01$) and $-1.66 \pm 0.33$ AU ($P < 0.01$) without affecting the time to negative peak ($0.71 \pm 0.04, 0.77 \leq 0.07,$ and $0.77 \pm 0.07$ s for DMI0, DMI0.3, and DMI1.0, respectively). Desipramine also attenuated the steady-state response from $-1.01 \pm 0.23$ to $-0.56 \pm 0.15$ ($P < 0.05$) and $-0.58 \pm 0.09$ AU ($P < 0.05$).
Figure 3A shows the group-averaged $H_{SNA-AP}$ obtained from protocol 1. The transfer gain decreased as the frequency increased in each condition, indicating low-pass characteristics of the baroreflex peripheral arc. The sharp peak around 0.5–0.6 Hz corresponds to the artificial ventilation frequency. The gain plot and steepened the decreasing slope of transfer gain. The phase approached zero radians at each portion in the gain plot and steepened the decreasing slope of transfer gain at the lowest frequency. The phase approached zero radians at the lowest frequency. The phase at 0.1 Hz lagged more in DMI0.3 and DMI1.0 compared with DMI0. The phase at 0.1 Hz lagged more in DMI0.3 and DMI1.0 compared with DMI0. The coherence values were 0.72 ± 0.07, 0.64 ± 0.08, and 0.50 ± 0.08 under the DMI0, DMI0.3, and DMI1.0 conditions, respectively. The averaged coherence did not differ among the three conditions.

Figure 3B shows the AP step response to unit changes in SNA calculated from $H_{SNA-AP}$. Desipramine did not affect the steady-state response (1.00 ± 0.06, 1.00 ± 0.13, and 0.93 ± 0.12 mmHg under DMI0, DMI0.3, and DMI1.0, respectively). The rise times of the step response were 12.4 ± 1.2, 14.2 ± 0.8, and 15.8 ± 0.6 s, respectively. The rise time was significantly longer in DMI1.0 than in DMI0 ($P < 0.05$).

Figure 4A shows the group-averaged $H_{SNA-HR}$ obtained from protocol 1. The transfer gain decreased as the frequency increased in each condition. Although desipramine did not affect the transfer gain at the lowest frequency, it narrowed the flat portion in the gain plot and steepened the decreasing slope of transfer gain. The phase approached zero radians at the lowest frequency. The phase at 0.1 Hz lagged more in DMI0.3 and DMI1.0 than in DMI0. The coherence values were 0.72 ± 0.07, 0.64 ± 0.07, and 0.50 ± 0.08 under the DMI0, DMI0.3, and DMI1.0 conditions, respectively. The averaged coherence was significantly smaller in DMI1.0 than in DMI0 ($P < 0.05$).

Figure 4B shows the HR step response to unit changes in SNA calculated from $H_{SNA-HR}$. Desipramine did not affect the steady-state response (1.00 ± 0.05, 1.21 ± 0.11, and 0.98 ± 0.08 beats/min, respectively). Desipramine prolonged the rise time of the step response from 10.2 ± 1.7 to 13.2 ± 1.4 ($P < 0.05$) and 16.2 ± 0.9 s ($P < 0.01$).

Figure 5A shows the group-averaged $H_{CSP-AP}$ obtained from protocol 1. The transfer gain decreased as the frequency increased in each condition. Desipramine decreased the transfer gain in DMI0.3 and DMI1.0 compared with DMI0. The phase approached π radians at the lowest frequency, reflecting the negative feedback attained by the total baroreflex loop. The coherence values were 0.50 ± 0.06, 0.50 ± 0.05, and 0.43 ± 0.06 under the DMI0, DMI0.3, and DMI1.0 conditions, respectively. The averaged coherence was significantly smaller in DMI1.0 than in DMI0 ($P < 0.05$).

Figure 5B shows the AP step response corresponding to $H_{CSP-AP}$. Desipramine attenuated the steady-state response from −1.00 ± 0.06 to −0.58 ± 0.16 ($P < 0.05$) and −0.43 ± 0.10 ($P < 0.01$). Desipramine did not affect the rise time of the step response (10.8 ± 2.0, 13.5 ± 2.4, and 13.1 ± 2.7 s for DMI0, DMI0.3, and DMI1.0, respectively).

### Table 1. Mean levels of CSP, SNA, AP, and HR in protocol 1

<table>
<thead>
<tr>
<th>Condition</th>
<th>CSP (mmHg)</th>
<th>SNA (AU)</th>
<th>AP (mmHg)</th>
<th>HR (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMI0</td>
<td>92 ± 5</td>
<td>105 ± 1</td>
<td>89 ± 10</td>
<td>277 ± 17</td>
</tr>
<tr>
<td>DMI0.3</td>
<td>92 ± 5</td>
<td>72 ± 4</td>
<td>92 ± 9</td>
<td>289 ± 17†</td>
</tr>
<tr>
<td>DMI1.0</td>
<td>92 ± 5</td>
<td>52 ± 5†</td>
<td>83 ± 10</td>
<td>288 ± 18*</td>
</tr>
</tbody>
</table>

Data are means ± SE. CSP, intracarotid sinus pressure; SNA, sympathetic nerve activity; AP, arterial pressure; HR, heart rate; DMI0, control conditions; DMI0.3 and DMI1.0, after iv bolus injections of 0.3 and 1.0 mg/kg desipramine, respectively. *$P < 0.05$, †$P < 0.01$ vs. DMI0 by Dunnett’s test.
Table 2 summarizes the parameters of transfer functions obtained from protocol 1. In \( H_{CSP-SNA} \), the steady-state gain was significantly smaller in DMI\_0.3 and DMI\_1.0 than in DMI\_0. The two corner frequencies and the pure delay did not change among the three conditions. In \( H_{SNA-AP} \), there were no significant differences in the steady-state gain among the three conditions. The natural frequency decreased to 74 ± 7% and 71 ± 7% in DMI\_0.3 and DMI\_1.0, respectively, compared with...
DMI₀. The damping ratio did not differ among the three conditions. The pure delay was significantly prolonged by desipramine in DMI₁₀. In $H_{\text{SNA-HR}}$, there were no statistical differences in the steady-state gain among the three conditions. The natural frequency was lower in both DMI₀.₃ (47 ± 4%) and DMI₁₀ (36 ± 2%) than in DMI₀. The damping ratio was significantly decreased in both DMI₀.₃ and DMI₁₀. The pure delay was significantly prolonged in DMI₁₀. In $H_{\text{CSP-AP}}$, the

Fig. 4. A: transfer functions from SNA to HR obtained from the DMI₀, DMI₀.₃ and DMI₁₀ protocols in protocol 1. Gain plots, phase plots, and coherence functions are shown. Desipramine administration decreased the transfer gain at high frequencies. The effects of desipramine on the gain plot were much more evident than in Fig. 3. Dashed oblique lines in the gain plots represent a slope of −40 dB/decade. B: step responses in HR derived from the transfer functions from SNA to HR. Desipramine slowed the HR step response. Solid and dashed lines represent mean and mean ± SE values, respectively. ΔHR, change in HR.

Fig. 5. A: total baroreflex loop transfer functions from CSP to AP obtained from the DMI₀, DMI₀.₃ and DMI₁₀ protocols in protocol 1. Gain plots, phase plots, and coherence functions are shown. Desipramine administration decreased the transfer gain. B: step responses in AP derived from the total baroreflex loop transfer functions. Desipramine attenuated the steady-state AP response. Solid and dashed lines represent mean and mean ± SE values, respectively.
transfer gain was significantly decreased by desipramine. The corner frequency and pure delay did not differ among the three conditions. Upon comparing H_{SNAP-HR} and H_{SNAP-AP} under the DMI0 condition, the natural frequency was significantly higher, the damping ratio was significantly greater, and the pure delay was significantly shorter in H_{SNAP-HR} than in H_{SNAP-AP}.

Figure 6 summarizes the time control for the transfer function analysis obtained from protocol 2. Figure 6, A–D, illustrates group-averaged H_{CSP-SNA}, H_{CSP-AP}, H_{SNAP-HR}, and H_{CSP-AP}, respectively. In Fig. 6, A–D, the transfer functions were calculated from the first (left) and second (right) recordings with an intervening interval of 40 min. There were no significant differences in the fitted parameters between the two recordings in each of the transfer functions (Table 3).

**DISCUSSION**

We have examined the effects of neuronal NE uptake blockade on dynamic characteristics of the carotid sinus baroreflex. Intravenous desipramine administration decreased dynamic gain of the baroreflex neural arc, resulting in decreased dynamic gain of the total baroreflex. Natural frequency of the low-pass filter was significantly decreased by desipramine in both dynamic AP and HR responses to SNA, but to a much greater extent in the latter than the former.

**Effects of desipramine on dynamic characteristics of the baroreflex neural and peripheral arcs.** Intravenous desipramine administration decreased mean SNA, consistent with previous studies (5, 11, 12) (Table 1). Although reflex changes in SNA induced by changes in AP should be taken into account under baroreflex closed-loop conditions, CSP was kept constant independent of AP in the present experimental settings. Thus the decrease in mean SNA was not secondary to changes in the baroreceptor pressure input. The noradrenergic neurons are located in ventral and dorsal columns in the medulla (A1 and A2 groups) (31). These neurons project to the hypothalamus and control cardiovascular and endocrine functions. In the pons the ventral column includes the A5 and A7 cell groups, which project to the spinal cord that modulates autonomic reflexes and pain sensation. The A6 cell group in the locus ceruleus has extensive projections to the cerebral cortex and cerebellum, as well as descending projections to the brain stem and spinal cord. The decreased NE clearance around these noradrenergic neurons after desipramine administration would increase the NE level in the brain stem, leading to the suppression of SNA via stimulation of the presynaptic inhibitory α2-adrenergic receptors (8). Although desipramine could inhibit sympathetic ganglionic transmission, the preganglionic SNA also decreased during desipramine administration in a previous study (8). Therefore, the reduction of renal SNA might be attributable to the central action of desipramine.

The present results suggest that, for the first time to our best knowledge, desipramine suppressed not only mean SNA but also the transfer gain from CSP to SNA (Fig. 2, Table 2). Changes in the transfer function were not resulting from the effects of elapsed time, as the transfer function did not differ between C1 and C2 in protocol 2 (Fig. 6, Table 3). The derivative characteristics of the baroreflex neural arc were preserved despite the significant loss of transfer gain under the desipramine treatment. The attenuation of SNA response to changes in CSP is in line with the human study by Tank et al. (36) wherein the neuronal NE uptake inhibition by reboxetine impaired the ability of vasomotor center to respond to sympathetic stimuli. Bertram et al. (5) demonstrated that intravenous desipramine administration almost halved dynamic gain of the transfer function from aortic depressor nerve stimulation to AP. However, whether the transfer function from aortic depressor nerve stimulation to SNA or that from SNA to AP was responsible for the reduced dynamic gain was undetermined in their study. We speculate that changes in the neural arc account for the desipramine-induced reduction of the baroreflex dynamic gain during aortic depressor nerve stimulation.

The steady-state gain of the baroreflex peripheral arc was not attenuated by desipramine (Fig. 3, Table 2), contrasting with the decreased transfer gain of the hindlimb vascular conductance response to electrical stimulation of the lumbar sympathetic chain reported by Bertram et al. (5). The preserved steady-state gain of the transfer function from SNA to HR after desipramine (Fig. 4, Table 2) was also inconsistent with our previous study (26), wherein intravenous desipramine decreased the transfer gain of the HR response to electrical stimulation of the cardiac sympathetic nerve. The apparent contradictions may be explained as follows. Intravenous desipramine decreases the transfer gain in H_{SNAP-AP} or H_{SNAP-HR} via the peripheral effect if mean SNA during the dynamic sympathetic perturbation is unchanged such as in the case of electrical stimulation experiments. However, mean SNA was significantly decreased by intravenous desipramine in the present experimental settings. According to previous studies, mean SNA during the dynamic sympathetic perturbation has significant influence on the transfer function from SNA to HR (4, 26); an increase in mean SNA decreases the transfer gain, and vice versa. An increase in mean SNA accumulates NE in the synaptic nuclei which is released into the brain stem when the drug is administered intravenously.

### Table 2. Effects of desipramine on transfer function parameters in protocol 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>DMI0</th>
<th>DMI1</th>
<th>DMI2</th>
</tr>
</thead>
<tbody>
<tr>
<td>$H_{CSP-SNA}$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$K_{AU/mmHg}$</td>
<td>1.03±0.09</td>
<td>0.79±0.09*</td>
<td>0.60±0.08†</td>
</tr>
<tr>
<td>$f_{c1}$, Hz</td>
<td>0.051±0.005</td>
<td>0.045±0.005</td>
<td>0.061±0.008</td>
</tr>
<tr>
<td>$f_{c2}$, Hz</td>
<td>0.52±0.07</td>
<td>0.50±0.05</td>
<td>0.54±0.07</td>
</tr>
<tr>
<td>L, s</td>
<td>0.19±0.01</td>
<td>0.24±0.04</td>
<td>0.20±0.03</td>
</tr>
<tr>
<td>Goodness of fit</td>
<td>0.88±0.03</td>
<td>0.82±0.04</td>
<td>0.82±0.03</td>
</tr>
</tbody>
</table>

Data are means ± SE. $H_{CSP-SNA}$, neural transfer function from CSP to SNA; $H_{SNAP-AP}$, peripheral transfer function from SNA to AP; $H_{SNAP-HR}$, transfer function from SNA to HR; $H_{CSP-AP}$, total baroreflex loop transfer function from CSP to AP; $K$, steady-state gain; $f_{c1}$ and $f_{c2}$, corner frequencies defining the derivative and high-cut characteristics; $f_{n}$, natural frequency; $L$, corner frequency; $L$, damping ratio; $L$, pure delay. *$P<0.05$, †$P<0.01$ vs. DMI0.
cleft, probably reducing relative changes in the NE concentration per nerve impulse and attenuating the dynamic response of a target organ. Activation of presynaptic \(\alpha_2\)-adrenergic receptors may also inhibit the amount of NE release per nerve impulse, reducing the transfer gain. The decrease in mean SNA via the central effect of desipramine, which itself is expected to increase the transfer gain, might have counteracted the inhibitory peripheral effect of desipramine, rendering the steady-state gain in \(H_{SNA-AP}\) or \(H_{SNA-HR}\) unchanged in the present study.

Differential effects of intravenous desipramine on the AP and HR responses. As shown in Figs. 3 and 4, low-pass characteristics of the dynamic sympathetic regulation differed between the AP and HR responses in the control (DMI 0) condition. The natural frequency was significantly higher and the damping ratio was significantly greater in \(H_{SNA-HR}\) than \(H_{SNA-AP}\), suggesting that HR responded more quickly to SNA than AP did under control conditions. Although intravenous desipramine lowered the natural frequency of both \(H_{SNA-HR}\) and \(H_{SNA-AP}\), the effects of desipramine were much stronger on \(H_{SNA-HR}\) than \(H_{SNA-AP}\). Because changes in HR could affect AP and because we did not pace the heart, changes in \(H_{SNA-AP}\) might in part result from changes in \(H_{SNA-HR}\). However, the relatively small change in the natural frequency of \(H_{SNA-AP}\) compared with \(H_{SNA-HR}\) suggests a limited influence of the dynamic HR response on the dynamic AP response. Liu et al. (24) provided a similar result that the contribution of HR to dynamic AP regulation was negligible in anesthetized rabbits. Because cardiac output is chiefly determined by venous return under conditions of normal cardiac function and normal HR ranges, changes in the dynamic HR response alone could not significantly affect the dynamic AP regulation.

The differential effects of neuronal NE uptake blockade on the AP and HR responses have been explained by the fact that the synaptic cleft is narrower in the heart than in the vasculature (28). Because of these morphological differences, the removal of NE from the synaptic cleft is far more dependent on neuronal NE uptake in the heart than in the vascular bed (15). The effects of neuronal NE uptake blockade on the dynamic sympathetic regulation of target organs have been examined using electrical stimulation of the sympathetic nerves (5, 26) without taking into account the differences in the pattern and frequencies between electrical stimulation and native sympathetic discharge. In the present study, the differential effects of neuronal NE uptake blockade on dynamic AP and HR responses were confirmed using native sympathetic discharge.
The effects of desipramine on the dynamic vagal control of HR. Further studies are required to elucidate the tricyclic antidepressants, it could affect the vagal control faster than the sympathetic control. Although desipramine might have participated in the observed changes in the baroreflex dynamic characteristics. To address this question, further studies are required where carotid sinus nerve activity is measured during desipramine administration.

In conclusion, neuronal NE uptake blockade reduced the total baroreflex gain mainly through attenuation of the neural arc gain. The effects of desipramine differed between dynamic sympathetic controls of HR and AP as consistent with the results obtained by electrical stimulation of the sympathetic nerve; the HR response was more decelerated than the AP response by desipramine. In both HR and AP responses, however, the transfer gain at the lowest frequency was unaffected by desipramine, contrasting to the results obtained by electrical stimulation of the sympathetic nerve. The suppression of mean SNA via the central effects of desipramine might have prevented the complete saturation of NE kinetics at the synaptic cleft, preserving the transfer gain at a basal level in the lowest frequency. The diverse effects of neuronal NE uptake blockade observed here may underlie the complex pathophysiology in a subgroup of patients with orthostatic intolerance or essential hypertension related to neuronal NE uptake deficiency.

**APPENDIX**

**Mathematical basis for “white-noise analysis.”** We assume that a system under study is regarded as time invariant within a time window of interest. We also assume that the system is regarded as linear when the input range is limited around the operating point of the system. The output of such a linear time invariant system is expressed as

\[ y(t) = h(t) * x(t) + n(t) = \int_{-\infty}^{\infty} h(\tau)x(t-\tau)d\tau + n(t) \quad (A1) \]

where \( x(t) \) and \( y(t) \) are the input and output signals of the system, respectively; \( h(t) \) is the system impulse response; \( * \) denotes the convolution operator; \( \tau \) is a time variable; and \( n(t) \) represents noise in terms of a linear systems analysis. The noise may include physiological signal unrelated to the input, nonlinear system response, and physical noise in the signal measurement.

The frequency-domain representation of Eq. A1 is

\[ Y(f) = H(f)X(f) + N(f) \quad (A2) \]

where \( X(f) \), \( Y(f) \), \( N(f) \), and \( H(f) \) are Fourier transforms of \( x(t) \), \( y(t) \), \( n(t) \), and \( h(t) \), respectively. \( H(f) \) represents the system transfer function. The convolution operator in the time domain is changed to the algebraic multiplication in the frequency domain. Multiplying the complex conjugate of \( X(f) \) \( [X(f)]^* \) with both sides of Eq. A2 and ensemble averaging them over multiple segments yields

\[ E[Y(f)X(f)^*] = H(f)E[X(f)X(f)^*] + E[N(f)X(f)^*] \quad (A3) \]

where \( E[\cdot] \) indicates the ensemble average operation. Note that \( H(f) \) is time invariant and thus can be outside of the ensemble average operation. When \( X(f) \) is white noise, the cross-spectral term between \( N(f) \) and \( X(f) \) asymptotically vanishes with increasing the number of
ensemble average. Therefore, we can estimate the system transfer function even in the presence of significant noise using the following equation

$$H(f) = \frac{E[X(f)X(f)^*]}{E[X(f)]X(f)^*} = \frac{S_X(f)}{S_X(f)}$$

where $S_X(f)$ is the cross-spectral density function between input and output, and $S_X(f)$ is the autospectral (also called power spectral) density function of the input. Because $H(f)$ is a quotient, it is important to make $S_X(f)$ sufficiently large for accurate determination of $H(f)$ over the frequency range of interest. The white noise input, which is rich in frequency components, is the most appropriate for $H(f)$ determination.

White-noise analysis vs. other system identification methods. Theoretically, $h(t)$ corresponds to the system response obtained by an impulse input. The system step response, derived from the time integral of $h(t)$, can be measured from the system response to a step input. However, the system identification method using the impulse or step input based on a single experimental trial is susceptible to unintentional noise in the output signal commonly encountered in the physiological experiments. To reduce the noise effects, repeated measurements of the system response to an identical stimulus should be averaged. In addition, an exact impulse or step input is unrealizable in many circumstances. To correct the deviation of the actual input from the ideal input, the measured system response should be deconvolved with the actual input signal, making the system identification procedure more complex than the ideal case. Taken together, measuring the system response to the impulse or step input may not necessarily be better or easier than the white noise analysis in terms of the required experimental duration and the computational burden when considering actual applications.

Other system identification methods frequently used are time-domain parametric estimation approaches such as an autoregressive moving average model. The parametric system identification methods are dependent on the model specification. If the order of the model is much greater than that of a system under study, the model becomes susceptible to subtle noises in measurements that are not actual system response. If the order of the model is much less than that of a system, the model lacks the freedom for accurate system description. The sampling interval also has a great influence on the system identification. Therefore, the model order and sampling interval for the time-domain parametric estimation should be specified based on some knowledge of the system being analyzed. In contrast, the nonparametric frequency-domain analysis employed in the present study does not impose any assumptions on the system characteristics except for the linearity. Therefore, the white-noise analysis may be most suitable for exploring changes in system characteristics.

GRANTS

This study was supported by Health and Labour Sciences Research Grant for Research on Advanced Medical Technology from the Ministry of Health Labour and Welfare of Japan (13004001, H14-Nano-002), by Grant-in-Aid for Scientific Research (A 15200040, C 15500786) from the Japan Society for the Promotion of Science, and by the Program for Promotion of Fundamental Studies in Health Science of the Organization for Pharmaceutical Safety and Research of Japan.

REFERENCES