Dynamics of heart rate response to sympathetic nerve stimulation

ABDELKADER MOKRANE AND RÉGINALD NADEAU

Research Center, Hôpital du Sacré-Cœur de Montréal, and Department of Medicine, Faculty of Medicine, Université de Montréal, Montreal, Quebec, Canada H4J 1C5

Mokrane, Abdelkader, and Réginald Nadeau. Dynamics of heart rate response to sympathetic nerve stimulation. Am. J. Physiol. 275 (Heart Circ. Physiol. 44): H995–H1001, 1998.—Electrical stimulation of the right cardiac sympathetic nerve was used to achieve a step increase of norepinephrine concentration at the sinus node. The heart rate (HR) response to sympathetic stimulation was characterized by a first-order process with a time delay. For moderate to high intensities of stimulation the mean delay and time constant were 0.7 and 2.1 s, respectively, and for low intensities of stimulation they were 0.4 and 1.1 s, respectively. From the analysis of the HR response to different patterns of nerve stimulation, in vivo neurotransmitter kinetics were estimated. The time constant of norepinephrine dissipation averaged ~9 s. These results combined with computer simulations revealed two facets of sympathetic neural control of HR: 1) negligible role of the sympathetic system in beat-to-beat regulation of HR under stationary conditions and 2) ability of HR to react relatively quickly (within a few seconds) to sharp increases in sympathetic nerve traffic.

Since the early investigations of the sympathetic nervous system, it has been observed that the heart rate (HR) step response to sympathetic nerve stimulation is characterized by a time delay of 1–3 s followed by a slow increase with a time constant of 10–20 s (25). HR decay after nerve stimulation cessation is even slower and is generally preceded by a longer time delay. In the frequency domain the HR response to sympathetic input fluctuations was characterized by a low-pass filter system with a cutoff frequency between 0.01 and 0.02 Hz coupled to a 1.7-s delay (1).

The sympathetic control of HR involves a cascade of biochemical and biophysical processes (9, 11, 14): 1) Norepinephrine (NE) released at sympathetic nerve terminals is removed from the neuroeffector space mainly through reuptake by nerve terminals and washout by the coronary circulation. 2) NE binds to postsynaptic β-adrenergic receptors, which are linked to stimulatory guanosine nucleotide-binding (Gt) proteins. 3) Ionic current channels are activated mainly through a second messenger pathway, namely, adenylate cyclase and cAMP systems. The slow development of the chronotropic response has been attributed to the slow NE dissipation rate and/or to the sluggishness of the intracellular adrenergic system (19). However, the relative contribution of each subsystem to the overall dynamic response has not been quantified in vivo. Moreover, because NE is the first link in the sympathetic neuroeffector transmission, its slow kinetics could mask the expression of the postsynaptic adrenergic activity.

In this study we used a sympathetic nerve stimulation protocol to simulate a step increase of NE concentration ([NE]) at the sinus node with the aim of characterizing the dynamics of the HR response and gaining insight into the mechanisms of sympathetic neural control of HR.

METHODS

Animal preparation. Eight adult mongrel dogs, weighing 18–30 kg, were anesthetized with α-chloralose (80 mg/kg iv) and artificially ventilated with room air. Additional doses of anesthetic were given regularly to maintain an appropriate level of anesthesia. The right femoral vein and the right femoral artery were cannulated for the infusion of drugs and for the monitoring of arterial blood pressure. The right stellate ganglion was isolated and decentralized. A pair of electrodes was attached to the right ansa subclavia or the right stellate ganglion for stimulation. Nerve stimulations were performed using a programmable stimulator (BM-SCP, Institut de génie biomédical, Montreal, PQ, Canada) coupled to a constant-current unit. Supramaximal stimulatory current pulses (2–4 mA) of 2-ms duration each were applied. Finally, a pair of electrodes was implanted on the right atrial epicardium to record an atrial electrogram. Atropine (0.2 mg/kg) was injected intravenously to block parasympathetic effects.

Stimulation protocol. To analyze the HR response to a step increase of [NE] at the sinus node, we used the stimulation protocol illustrated in Fig. 1A. At the start of the stimulation, a rapid increase of [NE] is obtained by delivering an impulse train. [NE] is then maintained at a constant level by application of a constant-frequency pulse stimulation. However, to obtain a steplike increase of [NE], the initial increase of [NE] should match the [NE] reached at the steady state. Because the NE removal rate is unknown, the number of impulses per train must be adjusted by trial and error to obtain as close a match as possible. With the assumption that the HR response to NE stimulation is a first-order process, a steplike increase of [NE] should induce a monoexponential HR increase. On the basis of this assumption, an overshoot (undershoot) observed in the HR response should indicate that the number of initial impulses per train is overestimated (underestimated) (Fig. 1B). Consequently, the optimal number of impulses per train was chosen so that only a negligible overshoot or undershoot could be observed in the HR response.

Data analysis. HR increase and decay were fitted by time-delay monoeponential functions using a Levenberg-Marquardt nonlinear regression algorithm (21) provided by the SigmaPlot software package (Jandel Scientific)

\[
y(t) = \Delta HR \left(1 - e^{-(t-d)HR\tau_e}\right) \tag{1}
\]

for HR increase and

\[
y(t) = \Delta HR e^{-(t-d)HR\tau_e} \tag{2}
\]

for HR decay, where \(y(t)\) is the HR time increase or decay and \(\tau_{HR}, d\), and \(\Delta HR\) represent the unknown time constant, time
delay, and steady-state amplitude to be estimated, respectively. These parameters were estimated for four levels of sympathetic nerve stimulation: 0.5, 1, 2, and 4 Hz.

For comparison between groups, statistical analyses were carried out by means of Student’s paired t-test. \( P < 0.05 \) was considered significant. Averaged data are given as means ± SE.

RESULTS

Parameters of NE kinetics. A step increase of \([\text{NE}]\) is obtained when the initial rise of \([\text{NE}]\) (left-hand side of Eq. 3) equals the mean steady-state \([\text{NE}]\) (right-hand side of Eq. 3)

\[
Nq = q_{\text{NE}} f
\]  

(3)

where \( N \) is the number of train impulses, \( q \) is the \([\text{NE}]\) quantum released by one stimulatory pulse, \( t_{\text{NE}} \) is the time constant of the NE removal process, and \( f \) is the steady-state sympathetic stimulation frequency. The right-hand side of Eq. 3 was obtained by integrating steady-state \([\text{NE}]\) over one heart period. Computational details are given in the APPENDIX.

From Eq. 3, the time constant of the removal process was estimated by

\[
\tau_{\text{NE}} = \frac{N}{f}
\]  

(4)

where \( \tau_{\text{NE}} \) values ranged from 7 to 12 s and averaged 9.1 ± 1.9 s.

Dynamic parameters of the postsynaptic noradrenergic response. The HR response to a step-like increase of \([\text{NE}]\) was adequately fitted by a time-delay monoexponential rise. The time constants and delays of HR increase were roughly constant for frequencies of sympathetic nerve stimulation \( \geq 1 \) Hz (1, 2, and 4 Hz) and averaged 2.1 ± 0.26 and 0.7 ± 0.09 s, respectively (Fig. 2A). However, these parameters were significantly lower for 0.5-Hz stimulation intensity (0.5 vs. 1 and 2 Hz) and averaged 1.1 ± 0.32 and 0.44 ± 0.11 s, respectively. The average magnitude of the steady-state HR increase (\( \Delta HR \)) vs. frequency of sympathetic stimulation was fitted by the Hill equation (Fig. 2B)

\[
\Delta HR = \frac{\Delta HR_{\text{max}} f^n}{K_f^n + f^n}
\]  

(5)
where \( f \) is sympathetic stimulation frequency, \( \Delta HR_{\text{max}} \) is the maximum value of \( \Delta HR \), \( K_f \) is the stimulation frequency producing a half-maximum response, and \( n \) is the Hill coefficient. \( \Delta HR_{\text{max}} \) and \( K_f \) were 72.6 beats/min and 1.21 Hz, respectively; \( n \) was 1.97.

Mathematical model. Considering the results obtained above, we propose a mathematical model of the sympathetic neural control of HR. The model is composed of a cascade of two functional blocks: 1) NE kinetics and 2) postsynaptic noradrenergic dynamics.

NE kinetics are described by a first-order process

\[
\frac{d[\text{NE}]}{dt} + \alpha_{\text{NE}}[\text{NE}] = q \sum \delta(t_i)
\]  

(6)

where \( \alpha_{\text{NE}} \) is the NE elimination rate, \( q \) is the [NE] quantum released by one stimulatory pulse, and \( \delta(t_i) \) is the Dirac impulsion corresponding to stimulation time occurrences \( t_i \).

Because absolute values of [NE] are unknown, \( q \) can be fixed to 1 without loss of generality.

Postsynaptic dynamics are given by

\[
\frac{\partial hr(t, [\text{NE}])}{\partial t} = -\alpha_{HR}([G([\text{NE}](t - d))] - hr(t, [\text{NE}])
\]  

(7)

where \( hr \) is the HR variation, \( \alpha_{HR} \) is the variation rate, \( d \) is the time delay of the HR response, and \( G \) is the steady-state HR response. According to Eq. 5 and noting that \( [\text{NE}] \) is linearly related to \( f \) (see Eq. A7), the steady-state HR response is obtained by

\[
\Delta HR = G([\text{NE}]) = \frac{\Delta HR_{\text{max}}[\text{NE}]}{K_f^2 + [\text{NE}]^2}
\]  

(8)

where \( K_{\text{NE}} \) is the [NE] producing a half-maximum response. The Hill coefficient was rounded to 2. According to the right-hand side of Eq. 3 and with the assumption that \( q = 1 \), it can be shown that

\[
K_{\text{NE}} = K_f r_{\text{NE}}
\]  

(9)

Time-domain simulation. We used the mathematical model described above to simulate the HR response to a squarelike function of the sympathetic nerve stimulation frequency. Figure 3C illustrates a comparison between experimental and simulated data. Simulated data fit more accurately the foot of the HR onset than does a time-delay monoexponential curve fitting. In some cases (not shown) the simulated HR increase was slightly faster, particularly during the second half of the increase. The theoretical [NE] time course is illustrated in Fig. 3B. The model fitted well the first half of HR decay but failed to fit its tail.

Frequency transfer function models. Equations 6–8 are nonlinear, and they describe completely the response of HR and NE to sympathetic nerve stimulation whatever the dynamic range of the input stimulation. However, it is also interesting to analyze the behavior of the system under stationary conditions, where input perturbations are small. This analysis could help in understanding the mechanisms governing HR variability, an important topic in which there was a large interest in the last decade (18). We used the transfer function approach to analyze the input vs. output relationships in the frequency domain, since it has been found to be a useful tool to analyze the linear dynamics of a given system (1, 22). A band-limited Gaussian white noise was used to simulate small sympathetic input perturbations. We simulated two types of input perturbations: 1) modulation of the nerve stimulation frequency (1) and 2) beat-to-beat modulation of stimulatory pulse duration. In the second mode of stimulation the sympathetic stimulatory pulses are synchronized to the heart period (phase-coupled stimulation), and we suppose that beat-to-beat modulation of NE release is achieved by varying pulse duration (a 2-ms pulse releases one maximal quantum of NE). Small signal noise response simulations based on linear approximations of the mathematical model described above (Eqs. 6–8) were carried out for different intensities of input nerve stimulation (operating points). Frequency transfer functions between output and input variables were estimated using spectral analysis methods (1, 22).
The transfer function magnitude and phase corresponding to the different input vs. output combinations, i.e., sympathetic stimulation vs. [NE], [NE] vs. HR, and sympathetic stimulation vs. HR, for both modes of stimulation are shown in Figs. 4 and 5 (for simplicity, we used the mean dynamic parameters corresponding to moderate and high stimulation intensities only). In both modes, corner frequencies of the low-pass filter characteristics of the different input vs. output combinations are similar. Cutoff frequencies corresponding to nerve input vs. [NE] output and [NE] input vs. HR output were 0.017 and 0.075 Hz, respectively. The HR response to nerve stimulation acts as a second-order filter with a 0.7-s delay resulting from a cascade of two low-pass filters. In the synchronized mode of stimulation the gain of the [NE] response to nerve input increases with the intensity of the sympathetic stimulation (Fig. 5A), whereas in the nonsynchronized mode the gain remains constant (Fig. 4A). The increase of the gain in the synchronized mode is attributable to the fact that the accumulated [NE] increases with the HR (see Eq. A7, where the mean stimulation frequency is HR/60). Figure 4C shows that the gain of the HR response to variations of the stimulation frequency decreases when the intensity of sympathetic stimulation increases. However, a close examination of the dynamic gain curve (gain vs. stimulation frequency), obtained by a frequency derivation of Eq. 5, shows biphasic characteristics (Fig. 6). The gain increases from zero, then decreases when the stimulation frequency increases. The maximum gain is obtained at a frequency of 0.7 Hz. In the synchronized mode of stimulation the gain of the HR response to nerve input decreases less rapidly when the stimulation intensity increases (Fig. 5C) because of the increasing gain of the NE response to nerve input (Fig. 5A).

**DISCUSSION**

By using an original sympathetic nerve stimulation protocol, we have been able to propose a key mechanism of sympathetic neural control of HR. From the analysis of HR dynamics, two components were indirectly characterized: the first was attributed to the neurotransmitter removal process at the presynaptic level, and the second was related to the dynamics of the intracellular response process. To our knowledge, this is the first attempt to quantify postsynaptic noradrenergic activity in vivo. A surprising result was that the β-adrenergic pathway responded faster than expected to sympathetic stimulation. Maximum mean time delay and time constant were 0.7 and 2.1 s, respectively.

Different studies on the β-adrenergic regulation of neurotransmitter-sensitive currents underlying the
pacemaking activity of sinus node cells, namely, the hyperpolarized-activated current (I_f) and the long-lasting calcium current (I_{Ca}), indicate that the major action of β-adrenergic stimulation occurs via a slow cytoplasmic pathway involving adenylate cyclase and cAMP activities (3, 15, 27). However, a faster pathway involving a direct G protein-mediated mechanism has also been demonstrated (2, 24, 29). Yatani and Brown (29) reported that the mean time constants of the I_{Ca}-fast and I_{Ca}-slow pathways were 150 ms and 36 s, respectively. In another study, the same authors reported a mean time constant of 570 ms of the I_f-fast pathway (30). In an effort to investigate the I_f-slow pathway mechanisms, DiFrancesco and Tortora (7) revealed that, in contrast to the I_{Ca}, I_f activation by cAMP involves a direct, phosphorylation-independent interaction with the ionic channels. They found that the action of cAMP on I_f induced a relatively fast response (~5 s). The mean time constant of the β-adrenergic response obtained in this study fits well within the range of the reported time constants of I_f-fast and I_f-slow pathways. Moreover, because the β-adrenergic response was faster at lower intensities of sympathetic stimulation than at higher intensities, it is possible that low β-adrenergic stimulation intensities favor the fast cellular pathway, whereas higher intensities favor the slow pathway. Other proofs of the existence of two different adrenergic pathways have been reported by Choate et al. (5). Their conclusion was based on the observation that two distinct components were involved in the generation of pacemaker action potential during sympathetic nerve stimulation.

It has been demonstrated that the cellular muscarinic-cholinergic signaling activity exhibits similar dual-pathway characteristics (2). In their analysis of the HR response to synchronized vagal stimulation, Mokrane et al. (22) speculated that the slow component of the

Fig. 5. Transfer function curves: beat-to-beat modulation of NE release. A–C as described in Fig. 4 legend.

Fig. 6. Calculated gain from nerve input to HR output as a function of nerve stimulation frequency. Dashed line, position of maximum gain.
vagally induced HR response could be related to the slow cholinergic pathway. Interestingly, the time constant of the slow component of the vagally induced HR response (2.5 s) is close to the time constant of the β-adrenergic-related component of HR revealed in this study.

It has been observed that sympathetic nerve discharge patterns can be highly irregular (13, 17, 20). Generally, nerve impulses are grouped in short bursts separated by long silent periods. Intraburst instantaneous frequency can be as high as 50 Hz, although the average nerve discharge frequency rarely exceeds 3 Hz under basal conditions (20). It has been suggested that this physiological erratic pattern may play a critical role in vasoconstriction activity (23). Our results favor the view that the sympathetic system is fast enough to respond to high-frequency burst-like sympathetic nerve traffic.

Another important finding of this work is the fact that the concentration-response relationship of NE-induced HR increase was characterized by a sigmoidal-type function with a Hill factor of 2, suggesting a positive cooperative agonist-receptor coupling process (2:1 binding).

It is well established that the neural reuptake (uptake 1) is the major process for removing NE within the neuroeffector junction (8, 12). Between 70 and 95% of the NE released by cardiac sympathetic nerves is recaptured. The remaining NE is metabolized in surrounding extraneurial tissues (uptake 2) (10, 16) and diffused into the bloodstream. Because of the difficulties inherent in the estimation of [NE] within the synaptic clefts, few data are available on the time constants of NE elimination. We report the study of Cousineau et al. (6), in which a complex kinetics model was used in in vivo tracer NE dilution experiments. Their estimated mean time constants for neural reuptake of NE and NE diffusion through the capillaries were 2.5 and 14 s, respectively. On the basis of these observations, one can speculate that the time constant of 9 s for NE dissipation obtained in this study probably reflects the kinetics of the neural reuptake process.

From the estimated parameters of NE and β-adrenergic kinetics, we have developed a mathematical model and carried out simulations to test its validity. The model reproduced remarkably well the HR increase induced by a constant sympathetic nerve stimulation frequency, although in some cases the simulated rise was slightly faster. It is possible that in these cases the time constant of NE removal was underestimated. The model supports also the observation that the time delay of the HR decay after cessation of sympathetic nerve stimulation increases with the intensity of the stimulation (28) (these observations were also confirmed in this study). This characteristic is attributable to the saturation of the HR vs. [NE] response curve at high intensities of stimulation. Unfortunately, the model failed to reproduce the tail of the HR decay and tended to overestimate the decline rate, although the simulated curve fitted well the first half of HR decay. The slow decline observed in the terminal downslope of the experimental data could be attributed to a diminished efficiency of NE reuptake by the nerve endings (uptake 1). It is possible that when the release of NE stops, NE will diffuse away from the surface of the nerve endings into surrounding tissues, favoring gradually the slower NE dissipation system (uptake 2). An intracellular mechanism can also be a factor of the slow HR decline. For example, the recovery process of the adrenergic system could be slower than the initiation process.

Simulated frequency transfer function magnitudes and phases between HR output variations and fluctuations of the sympathetic nerve stimulation frequency matched those obtained experimentally by Berger et al. (1). However, a new dimension has been added in this study to the interpretation of the transfer function curves: what was considered a low-pass filter with a cutoff frequency between 0.01 and 0.02 Hz with a 1.7-s delay is, in fact, a cascade of two low-pass filters with cutoff frequencies of 0.017 and 0.075 Hz (0.13 Hz at low sympathetic tones) coupled to a 0.7-s delay (0.4 s at low sympathetic tones). On the basis of the observation that sympathetic nerve discharges exhibit predominantly a cardiac-related rhythm (4, 26), a synchronized mode of input nerve stimulation was considered. Basically, the filter characteristics of the HR response in this mode were comparable to those in the nonsynchronized mode, confirming the hypothesis that, under stationary conditions, the sympathetic system is unable to modulate HR on a beat-to-beat basis and that the slow NE kinetics are the main cause of this behavior (19). Our results support also the finding that the 0.1-Hz baroreflex-related HR oscillations observed in humans and some animals are not mediated by the sympathetic system (18).

In conclusion, it is important to underline that even if the sympathetic system plays a minor role in short-term regulation of HR under stationary environments, it is fast enough to react within a few seconds to emergency situations such as an acute fall of arterial blood pressure and intense physical or emotional stress. The physiological significance of this response remains to be established, inasmuch as it represents a departure from the traditional role generally attributed to the sympathetic system.

APPENDIX

Determination of the mean steady-state [NE] obtained during constant sympathetic nerve stimulation frequency.

Given the time occurrences (t) of stimulation pulses, [NE] at time t (tNE(t)) is related to [NE] at time t-1 (tNE(t-1)) by the recurrence equation

\[ tNE(t) = q + tNE(t-1)e^{-\alpha_{NE}T} \]  \hspace{1cm} (A1)

with

\[ tNE(t_f) = q \]  \hspace{1cm} (A2)

where T is the time period between two successive pulses, t_f is the time occurrence of the first impulse, q is the [NE] quantum released by one pulse, and \( \alpha_{NE} \) is the NE elimina-
tion rate. Combining Eqs. A1 and A2, we obtain the geometric series
\[
[NE](t) = q(1 + e^{-\lambda T} + e^{-2\lambda T} + \ldots + e^{-n\lambda T}) \tag{A3}
\]
From Eq. A3 it can be shown that
\[
[NE](t) = \frac{1 - e^{-\lambda (t + 1)\lambda T}}{1 - e^{-\lambda \lambda T}} \tag{A4}
\]
[NE](t) at the steady state (t tends to infinity) is then
\[
[NE](t) = \frac{q}{1 - e^{-\lambda \lambda T}} \tag{A5}
\]
At time between \(t_{i-1}\) and \(t_i\), [NE](t) is given by
\[
[NE](t) = \frac{qe^{-\lambda \lambda (t - t_{i-1})}}{1 - e^{-\lambda \lambda T}} \tag{A6}
\]
The mean [NE] over one period \(T\) is then
\[
[N]E = \frac{q}{1 - e^{-\lambda \lambda T}} \int_{t_{i-1}}^{t_i} e^{-\lambda \lambda (t - t_{i-1})} dt = \frac{q}{\alpha NE T} = q_{NE} f \tag{A7}
\]
where \(q_{NE}\) is the time constant of NE dissipation and \(f\) is the stimulation frequency.

We are grateful to Pierre Fortier and Caroline Bouchard for excellent assistance in the experimental settings.

This work has been supported by the Medical Research Council of Canada. R. Nadeau is a Career Investigator of the Medical Research Council of Canada. Address for reprint requests: R. Nadeau, Research Center, Hôpital du Sacré-Cœur, Montreal, PQ, Canada H4J 1C5.

Received 13 August 1997; accepted in final form 11 May 1998.

REFERENCES