Frequency limits on aortic baroreceptor input to nucleus tractus solitarii

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Liu, Zhi, Chao-Yin Chen, and Ann C. Bonham. Frequency limits on aortic baroreceptor input to nucleus tractus solitarii. Am. J. Physiol. Heart Circ. Physiol. 278: H577–H585, 2000.—The frequency of baroreceptor volleys to the central nervous system can influence the fidelity of baroreceptor signal transmission and thus may affect baroreflex regulation of blood pressure. We examined 1) the extent to which frequency-dependent depression of aortic baroreceptor signals was initiated at the first central synapse between primary baroreceptor fibers and second-order nucleus tractus solitarii (NTS) neurons; 2) whether the pattern of baroreceptor input influenced the depression; and 3) the potential relevance to baroreflex sympathoinhibition. In urethane-anesthetized rats, NTS action potential response rates decreased (15), indicating that synaptic depression may limit baroreflex sympathoinhibition. Thus frequency limits on baroreceptor inputs at NTS synapses may affect baroreflex function.

synaptic transmission; in vivo; cardiovascular; aortic depressor nerve; lumbar sympathetic nerve

WITHIN THE CENTRAL NETWORK of the baroreflex pathway, baroreceptor information is integrated to regulate arterial blood pressure on a moment-to-moment basis. Much of the integration occurs in the nucleus tractus solitarii (NTS), where the baroreceptor signals are first processed (27). One aspect of the processing is that the frequency or timing of the baroreceptor inputs can influence the fidelity of synaptic transmission (12, 15, 17, 18, 23, 26). Seller and Illert (26) were the first to show that with increasing, yet physiologically relevant frequencies of carotid sinus nerve (CSN) input, there was a frequency-dependent depression of the postsynaptic responses; the amplitude of the evoked NTS potentials decayed by 50% with repetitive CSN stimulation at 10 Hz. Time-dependent depression, in which only two stimuli are applied, has also been observed for carotid nerve signal processing in the NTS. When a conditioning stimulus was followed by a test stimulus, as the interval separating the two was shortened, the NTS response to the test stimulus began to fail (12, 17, 23). In addition to such in vivo observations, studies performed in brain stem slices containing the NTS and primary autonomic afferent fibers in the solitary tract have also documented both frequency- and time-dependent synaptic depression (1, 5, 6, 13, 19, 28). Of particular relevance to the present work are the findings by Scheuer and colleagues (23) of time-dependent depression specifically of aortic baroreceptor signal transmission in vivo. We have recently demonstrated that with a repetitive barrage of aortic baroreceptor afferent signals to the NTS, there is also a frequency-dependent depression of signal transmission. When trains of 100 stimuli were delivered to the aortic depressor nerve (ADN) at increasing frequencies, the NTS action potential response rates decreased (15), similar to the amplitudes of carotid nerve-evoked synaptic potentials reported by Seller and Illert (26).

There remain, however, a number of questions about frequency-dependent depression of aortic baroreceptor signal transmission which, if answered, could provide new insight into the physiological relevance of this form of synaptic depression in baroreceptor afferent signaling and ultimately in baroreflex function. For example, what is the extent to which frequency-dependent depression occurs at the first central synapse? Do second-order NTS neurons serve simply as relayers of primary baroreceptor signals to more distal synapses for signal processing or does the processing begin at the first CNS synapse? Time-dependent depression of aortic baroreceptor signal transmission was shown to occur largely at distal synapses (23); however, the extent to which frequency-dependent depression occurs at second- or higher-order baroreceptor neurons is not known (15).

Most information on synaptic depression in the NTS in the intact animal has been obtained with constant frequency-repetitive stimulation or paired stimulus paradigms. However, many baroreceptor fibers provide a phasic input to the NTS synchronous with the systolic phase of the cardiac cycle rather than a constant input (7, 24, 25). Moreover, some, though not all, studies of reflex function suggest that phasic input patterns are more effective than fixed patterns in evoking baroreflex responses (7, 10, 11, 20). So, whether the pattern of
baroreceptor inputs influences the extent of frequency-dependent depression of baroreceptor signal transmission in the NTS is unknown.

Finally, whether there is any impact of frequency-dependent depression of baroreceptor signal transmission on baroreflex function is not clear. Seller and Illert (26) suggested that the frequency limitation for carotid sinus input to the NTS was manifest in baroreflex control of blood pressure, with maximal decreases in blood pressure occurring at 20–30 Hz CSN stimulation. Frequency limits on baroreflex function have also been reported in humans, although at higher frequencies (80 Hz) than reported earlier (3). Others (10, 11) have shown that maximal reflex responses are maintained at high stimulation frequencies, suggesting that frequency limits on signal transmission in the NTS are not conveyed to the reflex output. However, whether the maximal baroreflex responses would have been even larger in the absence of frequency limits in the NTS is unknown. Moreover, there are no studies in which NTS neuronal activity and reflex output have been simultaneously measured in real time during increasing frequencies of baroreflex volleys.

The purpose of this study was to answer three questions: 1) Is frequency-dependent depression of aortic signal transmission initiated at the first central synapse? 2) Does whether the ADN afferent volleys arrive in a phasic pattern (to mimic systolic input) or a constant pattern influence the extent of the depression? 3) Does the frequency-dependent depression in the NTS have any influence on the sympathetic component of baroreflex output? To answer these questions, we simultaneously measured NTS action potential responses of neurons classified as second or higher order and averaged lumbar sympathetic nerve activity (LSNA) responses during 100 ADN stimuli delivered in constant or phasic patterns at increasing frequencies. Using curve-fitting analysis, we predicted what impact frequency-dependent synaptic depression in the NTS might have on baroreflex-mediated sympathoinhibition.

**METHODS**

Experimental protocols followed in the work were approved by the Institutional Animal Care and Use Committee in compliance with the Animal Welfare Act and in accordance with Public Health Service Policy on Humane Care and Use of Laboratory Animals.

General animal preparation. Experiments were performed in male Sprague-Dawley rats, which were anesthetized with an initial injection of urethan (1.3 g/kg ip). Each rat was placed on a servo-controlled water blanket, and body temperature was monitored via a rectal temperature probe and maintained within 37 ± 1°C. The endotracheal tube was attached to the ventilator, and the rat was ventilated with oxygen-enriched air at 40–48 breaths/min. The ventilator rate was adjusted to maintain blood gases within normal limits. The inspiratory line of the ventilator was placed under 1–2 cmH2O to prevent atelectasis. A catheter was advanced through the femoral artery for measuring arterial blood pressure and withdrawing blood for blood gases and through the femoral vein for administering fluids and drugs. The electrocardiogram (ECG) was measured with surface electrodes. The ADN was approached via a cerebral lateral incision, placed on a bipolar silver hook electrode, covered with a mixture of petroleum jelly and mineral oil, and connected to a stimulus isolation unit. The lumbar sympathetic nerve trunk was accessed between L3 and L5 by a ventral abdominal approach. The nerve was placed on a bipolar silver hook electrode and covered with silicone gel (Wacker Sil-Gel 604) to isolate the electrode from the surrounding tissue and to ensure contact of the nerve and electrode.

The rats were placed in a stereotaxic frame and suspended by a thoracic vertebral clamp. An occipital craniotomy was performed. The caudal portion of the fourth ventricle was exposed by removing the dura and arachnoid membranes and then covered with warm mineral oil. At the start of the NTS recording period, the rats were paralyzed with gallamine (3 mg/kg iv). Anesthesia was maintained by administration of pentobarbital sodium (6.5 mg/kg iv) as needed. Before neuromuscular blockade, adequacy of anesthesia was determined every half-hour by pinching the hindlimb paw and monitoring for hindlimb flinch or withdrawal or observing a sudden fluctuation of mean arterial blood pressure (MABP) (>5 mmHg) or heart rate (HR) (>10%). During neuromuscular blockade, adequacy of anesthesia was tested every half-hour by determining whether there was a spontaneous or paw pinch-evoked fluctuation or increase (>5 mmHg) in MABP or increase (>10%) in HR.

 Extracellular recordings of single-unit NTS activity were made using a single-barrel glass pipettes filled with 2% Pontamine blue dye in 0.5 M sodium acetate. To search for ADN-activated neurons, the electrode was stereotaxically positioned on the dorsal surface of the brain stem over the NTS and lowered slowly as the ADN was activated at 0.6 Hz. Recordings were made on either side of the NTS but always ipsilateral to the ADN stimulated. From previous experience in searching for ADN-responsive neurons in the NTS (2, 8, 15), we limited the search to the region of the NTS extending in the rostrocaudal plane from 1.3 mm rostral to the level calamus scriptorius and from 0.2 to 1.4 mm lateral to midline. This region corresponds to the intermediate and caudal NTS where cardiovascular and respiratory afferent fiber terminals are concentrated (10). NTS single unit activity was fed via high-impedance source followers to second stage amplifiers and filtered between 0.3 and 3 kHz. LSNA signals were fed via a high-impedance source follower to a second stage amplifier and filtered between 10 and 3,000 Hz. For measuring ADN-evoked changes in averaged LSNA, the nerve activity was rectified and averaged using a stimulus-triggered averaging program (RC Electronics). Both raw and averaged LSNA were monitored during the experiment to assure viability and maximal depression in response to ADN stimulation. Single NTS unit activity, LSNA, arterial blood pressure, and ECG activity were fed in parallel to a thermal chart recorder, audiomonitor, and a digital tape recorder with a sampling rate of 11 kHz per channel for off-line analyses using RC Electronics.

NTS recording sites were marked by passing current (10 µA for 7 s every 15 s for 15 min, electrode negative) through the recording electrode to deposit the Pontamine dye. At the end of an experiment, the brain stem was removed and fixed in 4% paraformaldehyde and 10% sucrose. The brain stems were cut in 40-µm coronal sections, and the recording sites were verified histologically.

Protocols. The ADN was initially stimulated with constant current 0.5-ms pulses delivered at 20 Hz to determine the threshold current to produce a depressor response. For screening the NTS for ADN-activated neurons, the ADN was continuously stimulated at 0.6 Hz (0.5-ms pulses) with cur-
rents of at least three times the depressor threshold current as the recording electrode was lowered slowly through the nucleus. Once a neuron was isolated, we used the minimal ADN current that evoked NTS action potentials at a ~100% response rate at a stimulation frequency of 0.8 Hz. That current averaged 458 ± 352 μA. The same current was used to determine the onset latency and variability of the onset latency of the evoked responses and the action potential response rate at all frequencies. Viability of the ADN was tested throughout the experiment by stimulating at 20 Hz with currents at three times the depressor threshold and establishing that there was a decrease in arterial blood pressure. Each neuron was tested with two ADN stimuli separated by 5 ms; if it discharged an action potential to each of the two stimuli, it was classified as monosynaptically activated (19, 23). Trains of 100 ADN stimuli were delivered at frequencies of 0.8, 3, 6, 12, 24, and 48 Hz in a constant pattern. Frequencies were applied in random order. Each train of stimuli was separated by a 1-min interval. In 13 rats, we transected both vagi to determine whether vagal inputs affected the extent of the frequency-dependent depression of ADN signals; in 5 of those rats we also transected both ADN (peripheral to the stimulating electrode). There was no difference in the extent of the frequency-dependent depression in neurons studied with the ADN and vagi intact (n = 34) or without the vagi cut (n = 10) or with both ADN and vagi cut (n = 5), therefore the data were combined.

Because baroreceptor fibers can provide a pulse phasic input to NTS neurons synchronous with the systolic phase of the cardiac cycle (7, 24, 25), we compared frequency-dependent depression for phasic versus constant input patterns at the same mean frequencies. One hundred ADN stimuli were delivered in both phasic and constant patterns at mean frequencies of 12, 24, and 48 Hz. Phasic stimuli were delivered in cycles that corresponded to the R-R interval in the rat to mimic bursts of aortic baroreceptor activity (14). On the basis of a rat HR of 360 beats/min, the R-R interval averages 167 ms, with systole occurring within one-third of the interval (~56 ms, indicated by shaded area in Fig. 1). Thus, for a mean input frequency of 12, 24, or 48 Hz, the phasic input consisted of 2, 4, or 8 stimuli, respectively, applied in 56 ms (systole) every 167 ms (Fig. 1). The corresponding peak frequencies were 36, 72, or 144 Hz, respectively.

To compare the magnitude of the NTS action potential response rate and the averaged LSNA response in the same trials using the same frequencies, we used the ADN stimuli as triggers to simultaneously generate peristimulus time histograms of evoked NTS action potentials and averaged decreases in LSNA.

Data analysis. The number of action potentials evoked by ADN stimuli at each frequency was counted from peristimulus time histograms that were constructed from the 100 stimuli in each train (RC Electronics). The onset latency of the evoked responses and the variability of the onset latency were also determined from peristimulus time histograms. The onset latency and variability of the onset latency of NTS responses were compared in neurons classified as second-versus higher-order neurons using unpaired t-tests. To determine the response rate of the NTS neurons at each frequency, the number of action potentials evoked was expressed as a percentage of the 100 stimuli delivered. The effect of order on the frequency-dependent response rate of the NTS neurons was analyzed with a two-way repeated measures ANOVA with frequency as a within-subject effect and neuron order as a between-subject effect. The effect of pattern on the frequency-dependent responses was analyzed with a two-way repeated measures ANOVA with frequency as one within-subject effect and pattern as the other within-subject effect.

To examine the time course for the frequency-dependent decrease in NTS neuron responsiveness for each successive stimulus in the train of 100 stimuli, we counted the number of evoked action potentials and expressed that number as a percentage of the number of stimuli. The percentages were plotted for each successive stimulus at each frequency of ADN stimulation for both second- and higher-order neurons.

To determine the extent to which frequency-dependent depression in the NTS may affect the reflex decrease in LSNA for higher-order neurons showing at least a 15% synaptic depression for which we had simultaneous LSNA recordings, we multiplied each ADN input frequency by the average response rate (percent) of the NTS neuron at that frequency and designated the product as the NTS output frequency. We then fit the decrease in LSNA to the NTS output frequencies for each animal. From the curve fitting, we predicted the decrease in LSNA in the absence of frequency-dependent depression. We compared the predicted decrease in LSNA in the absence of frequency-dependent depression in the NTS with the actual decrease in LSNA measured with the frequency-dependent depression by using a two-way ANOVA with frequency as one within-subject effect and presence or absence of frequency-dependent depression as the other within-subject effect.

The peak amplitude and peak latency of the decreases in LSNA were determined by stimulus-triggered averages, constructed from the 100 stimuli in each train (RC Electronics). Each decrease in LSNA was expressed as a percentage of baseline activity immediately preceding the stimulus. The maximum depression and time constant for the frequency-dependent decreases in LSNA as a function of NTS output frequencies were determined by curve fitting. Data are expressed as means ± SE, unless otherwise indicated. Significance was claimed at P < 0.05.

RESULTS

The results were obtained in 29 rats. At the commencement of recording the mean arterial P02 was 197 ± 77 mmHg, Pco2 was 37.2 ± 5.9 mmHg, and pH was 7.41 ± 0.01 (means ± SD). Twenty-two recording sites (14 of second-order neurons and 8 of higher-order neurons) were histologically confirmed and were located in the intermediate NTS, where baroreceptor neurons are concentrated (16). All but three recordings were medial to the solitary tract. An example and the composite of the recording sites are shown in Fig. 2.

Frequency-dependent depression in NTS baroreceptor neurons. An example of frequency-dependent synaptic transmission in a second-order aortic baroreceptor...
A neuron is shown in Fig. 3. As shown in Fig. 3A, 100 ADN stimuli evoked action potential responses in a cell that met the criteria for a second-order neuron (discharged an action potential to two ADN stimuli separated by 5 ms, inset in Fig. 3A). The impulse activity had a cardiac rhythm as indicated by the R wave-triggered histogram of unit activity (Fig. 3B) and increased when arterial blood pressure was increased with 0.5 µg/kg iv phenylephrine (Fig. 3C). Frequency-dependent depression of the action potential response rate is shown in Fig. 3D.

The group data showing frequency-dependent depression in second- and higher-order aortic baroreceptor neurons are shown in Fig. 4. Frequency-dependent depression occurred in second-order neurons and to a greater extent in higher-order neurons (two-way ANOVA; *P < 0.0001, frequency; P < 0.0001, neuron order; no interaction). Fifteen of twenty-two second-order neurons and 28 of 30 higher-order neurons displayed at least a 15% decrease in the synaptic response at the highest frequency. All neurons were included in the analysis. Neurons that met the criteria for second-order had a mean onset latency of 6.7 ± 2.2 ms, significantly shorter than that of the higher-order neurons (20.8 ± 2.5 ms; P = 0.0004). In addition, the variability of the response onset was significantly shorter in the second- versus higher-order neurons (2.0 ± 0.5 ms vs. 14.0 ± 1.9 ms, respectively; P = 0.0001). Fourteen (6 second- and 8 higher-order) of the 52 neurons displayed a detectable cardiac rhythm.
however, it is possible that with higher blood pressures, more neurons would have exhibited a pulse-phasic firing pattern (27). Seven (4 second- and 3 higher-order) of 8 neurons tested were excited by increases in blood pressure induced by intravenous phenylephrine injections.

The time course of the frequency-dependent depression during successive stimuli at each frequency is shown for second- and higher-order neurons in Fig. 5. For second-order neurons, the synaptic depression at ADN input frequencies of 24 and 48 Hz did not commence until ~10 stimuli were applied and required about 40 stimuli to reach the maximum steady-state depression. For the higher-order neurons, starting at 6 Hz, the depression commenced after as few as two stimuli and required ~20 stimuli to reach the maximum steady state.

NTS and LSNA frequency-dependent responses. The frequency-dependent responses of NTS neurons and peak fall in averaged LSNA measured simultaneously are shown in Fig. 6 (n = 14). Figure 6A illustrates successive NTS action potentials, and the accompanying decrease in LSNA averaged over 100 ADN stimuli delivered at 0.8 Hz. The peak latency of the decrease in averaged LSNA was 174 ms; the mean peak latency for all LSNA responses was 163 ± 25 ms (means ± SD). The group data are shown in Fig. 6B. The NTS neurons (Fig. 6B, top) displayed the characteristic frequency-dependent depression (one-way repeated measures ANOVA, P < 0.0001). The amplitude of the decrease in averaged LSNA, expressed as a percentage of the baseline (Fig. 6B, bottom), was greater with the increasing frequencies of ADN stimulation (one-way repeated measures ANOVA, P < 0.0001).

The potential influence of frequency-dependent depression in the NTS on the reflex sympathoinhibition is shown in Fig. 7. The data are from seven animals with
simultaneous recordings of LSNA and of higher-order NTS neuron activity (to approximate NTS output). Shown in Fig. 7A, the measured decrease in averaged LSNA for each animal was plotted as a function of the calculated NTS output frequencies corresponding to ADN input frequencies of 0.8, 6, 12, 24, and 48 Hz. For each animal the decrease in LSNA was best fit to the NTS output frequencies with a single exponential. From the curve fitting for each animal, we determined the predicted decrease in LSNA if there was no frequency-dependent depression in the NTS; that is, when the NTS output frequency matched the ADN input frequency. Figure 7B shows 1) the measured decrease in averaged LSNA as a function of the ADN input frequency with the actual frequency-dependent depression observed in the NTS, and 2) the predicted decrease in averaged LSNA in the absence of frequency-dependent depression (when the NTS output frequency matched the ADN input frequency). The predicted decrease in LSNA in the absence of frequency-dependent depression was significantly greater than the measured decrease with frequency-dependent depression (two-way ANOVA; $P = 0.0487$, frequency; $P = 0.00001$, predicted vs. actual decrease in LSNA; no interaction).

**DISCUSSION**

The major findings of this study are that: 1) aortic baroreceptor signal transmission measured in the NTS in the whole animal is initiated at the first central synapse between the primary baroreceptor fibers and second-order neurons but is considerably more robust at more distal synapses; 2) calculations based on the simultaneous measurement of NTS and sympathetic output responses during increasing frequencies of ADN stimulation suggest that frequency-dependent depression of signal transmission in the NTS may modestly limit baroreflex-mediated sympathoinhibition; and 3) phasic patterns of aortic baroreceptor volleys, set to mimic systolic inputs, are marginally more effective in
eliciting frequency-dependent synaptic depression than constant patterns at the same mean frequencies but appear not to influence the sympathetic component of the reflex output.

The finding that frequency-dependent depression occurred at second-order neurons suggests that these neurons, rather than simply following primary baroreceptor inputs, have some signal conditioning capacity. These results confirm our previous observations of frequency-dependent depression at second-order neurons in the rabbit (15). Interestingly, Scheuer and colleagues (23) found that time-dependent depression of aortic baroreceptor signal transmission, observed when a conditioning and test stimulus were applied, whereas a consistent feature in higher-order neurons, did not generally occur in second-order neurons (23). The time course of the frequency-dependent depression in the current study reinforces the finding of Scheuer and co-workers (23) that when only two stimuli, there is no appreciable synaptic depression at second-order neurons (Fig. 5). In the present study, in general, the frequency-dependent depression at the second-order neurons did not begin until at least 10 volleys were delivered and required about 40 volleys for the maximum steady-state depression. By contrast at the higher-order neurons, the depression not only occurred with lower ADN input frequencies, but beginning with 6 Hz, the depression commenced with as few as two stimuli and required 20 stimuli to reach the steady-state depression (Fig. 5). These observations suggest that frequency-dependent depression requires a critical number of afferent volleys. This was also the case with our previous study in rabbit, wherein about 30 ADN stimuli were required to elicit the depression (15). The ADN stimulation frequencies associated with the frequency-dependent depression were within the physiological range of baroreceptor afferent activity; myelinated aortic baroreceptor activity increases to 40 Hz with arterial blood pressure increases of 13 mmHg and to 80 Hz with blood pressure increases of 28 mmHg (4).

The presumptive nature of the criteria for classifying neurons as second-order must be considered when interpreting the results of this study. Miles (19) emphasized the importance of establishing criteria beyond short-onset latency for distinguishing the activation of second- from higher-order neurons in describing frequency-dependent depression in the NTS. He used two criteria for presuming a cell was monosynaptically activated: 1) the ability to follow two stimuli separated by 5 ms; and 2) an onset variability of <0.5 ms for solitary tract-evoked excitatory postsynaptic potentials (EPSPs). The first criterion has been validated in vivo, insofar as NTS neurons identified independently as second-order (by anterograde tracing of the vagus nerve) reliably met the criterion of following two stimuli separated by 5 ms; however, the minimum latency variability was found to be a less reliable criterion (22). In the present study we relied on the first criterion; however, for cells that followed the two stimuli, the onset variability for the evoked response was 2.0 ± 0.5 ms, considerably less that that for neurons classified as higher order (14.3 ± 2.1 ms) and consistent with the presumption of a tighter onset variability of monosynaptically evoked responses. The variability was higher than that accepted by Miles (19) in vitro but similar to that reported by Scheuer et al. (23) for ADN-evoked action potentials in vivo (4 ± 1 ms). The method of measuring the postsynaptic responses may explain differences in onset variability; for example, EPSP onsets may vary less than the rate of rise of EPSPs initiating the action potentials. Thus estimates of latency variability that rely on action potential onsets may exceed those made by relying on EPSP or excitatory postsynaptic current onsets. The greater variability observed in vivo may also be explained by ongoing synaptic inputs originating from neurons outside the confines of the slice, which can have a fluctuating influence on the rate of rise of the EPSP and hence the appearance of the action potential. One other issue that may complicate the use of these criteria, particularly when measuring either EPSPs or action potentials, is the existence of extensive dendritic spines. To the extent that greater dendritic spine densities result in greater dendritic filtering, under conditions in which the membrane potential is not voltage-clamped, the dendritic filtering (21) might allow for a higher variability in the response latency and perhaps a failure to follow the twin stimuli, resulting in an erroneous classification of a second-order neuron as higher-order. Despite these considerations, the current findings are consistent with previous findings of frequency-dependent synaptic depression at second-order neurons in the NTS in rabbits (15) and in an in vitro NTS slice preparation (9). In the in vitro study, neurons met both presumptive criteria for classification as second order and were studied under voltage-clamp conditions, eliminating the confounding influences of postsynaptic changes in voltage-dependent ion channels and dendritic filtering.

The frequency-dependent depression of baroreceptor transmission was more robust in higher-order than in second-order neurons. At 48 Hz, the response rate was down approximately to 72% in second- and 30% in higher-order neurons. These findings confirm a trend suspected in our earlier study in the rabbit. In addition, in both studies the synaptic depression occurred in a larger proportion of higher-order than second-order neurons, occurring in 93% of higher-order compared with 68% in second-order neurons in this study and in 76% of higher-order compared with 64% in second-order neurons in the previous study in rabbit (15). These in vivo data, also in agreement with the in vitro voltage-clamping study (9), suggest that sequential NTS neurons in the baroreceptor afferent pathway may successively filter high frequency inputs before the signals are conveyed to distal synapses outside the NTS. The observation that at least some second-order neurons do not display frequency-dependent depression raises the possibility that some neurons in the baroreceptor afferent pathway are coded to safeguard signal transmission regardless of the frequency or number of afferent volleys.
The frequency limits in the NTS may simply serve as a low-pass filter, allowing incoming baroreceptor signals with a wide dynamic range (and hence less subject to noise) to be converted to signals having a smaller dynamic range (and hence more easily modulated by other inputs) within the NTS. If, however, the frequency-dependent depression is not counterbalanced by further processing at distal synapses outside the NTS, then these events in the NTS may influence the extent of baroreflex function. In this study, based on the curve-fitting data, there was a predicted diminution of the baroreflex-mediated sympatho-inhibition of 34% by the presence of frequency-dependent synaptic depression in the NTS (Fig. 7). The data raise the possibility that beyond simply filtering incoming baroreceptor signals, the frequency limits may curb the magnitude of reflex-mediated sympathoinhibition, perhaps damping excessive fluctuations in blood pressure.

Many baroreceptor fibers provide a pulse-phasic input to the NTS (7, 24, 25). In that regard, phasic patterns of CSN stimulation have been shown to be more effective than constant patterns in evoking baroreflex responses (7, 20), although, this has not been uniformly observed (10, 11). The present findings suggest that when comparing the mean input frequencies, a phasic pattern of baroreceptor volleys only marginally increases the extent of the frequency-dependent synaptic depression of baroreceptor signals and appears to have no significant effect on the sympathetic reflex output. On the other hand, a comparison of the peak instantaneous rather than the mean frequency of afferent volleys in the burst with the same fixed frequency suggests the opposite at least at one frequency. Given that we studied constant input frequencies up to 48 Hz by curve fitting the data, we could predict the extent of depression with an ADN input of 36 Hz, the peak frequency of the phasic input delivered with a mean frequency of 12 Hz. A comparison of the two patterns at the same peak frequency of 36 Hz showed no difference in the extent of synaptic depression at the second-order neurons (P > 0.05, paired t-test). However, for the higher-order neurons, the phasic input resulted in a greater depression (51 ± 10.8% from the phasic compared with 38.07 ± 0.9% from the constant input; P = 0.003, paired t-test). Ultimately, however, despite these two different statistical outcomes, the pattern of afferent input to the NTS, at least under these experimental conditions, did not affect the extent of the reflex sympathoinhibition, suggesting that the differences in synaptic depression in the NTS based on input pattern may not be important in the reflex output.

Whereas the phasic pattern of stimulation mimics systolic input, electrical stimulation, nonetheless, causes a synchronous firing of the afferent fibers that is not observed with the natural stimulus. So whereas the approach provides the advantages of reproducibly delivering and manipulating the patterns of stimuli, the nature and magnitude of the frequency-dependent depression of baroreceptor input in response to natural stimuli has yet to be determined. Still, the widespread observation of frequency or time depression of synaptic transmission in the NTS, both in the whole animal and in vitro (1, 5, 6, 13, 19, 28), suggests that the phenomenon may be physiologically important in modulating synaptic transmission of many autonomic signals conveyed to the NTS.

In conclusion, the results of this study suggest that when the same number of aortic baroreceptor afferent volleys arrive at second-order neurons in the NTS with increasing frequencies, synaptic events limit the frequencies of signals transmitted. These limits may not only serve to optimize synaptic transmission at proximal synapses in the baroreceptor afferent pathway but may also limit the magnitude of baroreflex sympathoinhibition, perhaps smoothing excessive fluctuations in baroreflex changes in arterial blood pressure.

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