What Does the Brain Know About Blood Pressure?

Steven W. Mifflin

Department of Pharmacology, The University of Texas Health Science Center at San Antonio, San Antonio, Texas 78229-3900

The integration of baroreceptor inputs within the central nervous system is modulated by a variety of inhibitory processes. It is proposed that, in hypertension, brain stem neurons adapt to increased excitatory baroreceptor inputs by increasing the efficacy of these inhibitory processes. Enhanced inhibition maintains some degree of reflex function in hypertension.

The arterial baroreceptor reflex is an important determinant of cardiovascular homeostasis. Stretch-sensitive mechano-receptors in the arteries relay information regarding the level and rate of change of blood pressure to the central nervous system, where it is processed to modulate the activity of neurohumoral factors in an attempt to minimize fluctuations in arterial pressure. Baroreceptor afferent inputs are initially integrated by neurons in the nucleus of the solitary tract (NTS), and these neurons then relay the processed input to other central sites involved in neurohumoral regulation of cardiovascular function. At the level of the hindbrain, these other central sites mediate baroreflex regulation of vagal outflow to the heart (via neurons in the nucleus ambiguous) and sympathetic outflow to the heart and blood vessels (via neurons in the caudal and rostral portions of the ventrolateral medulla).

It is clear that the peripheral and central components of the baroreceptor reflex can adapt in response to changes in physiological state. In chronic hypertension, these adaptations typically result in either normal or blunted baroreflex function. A reduced ability of the baroreflex to regulate arterial pressure would result in increased arterial pressure lability and could adversely affect cardiovascular function in hypertensive individuals. Therefore, adaptations that normalize baroreflex function around a higher arterial pressure, as observed in some hypertensive models, are of great potential significance. The past decade has seen a number of studies in both normotensive and hypertensive animals examining baroreceptor afferent integration within the brain stem. A number of integrative processes have been identified within the NTS that might contribute to central baroreflex adaptation in hypertension.

This brief review seeks to illustrate the importance of inhibitory mechanisms within the NTS and to speculate on the possible role of inhibitory mechanisms in mediating baroreflex adaptations in hypertension. The nature of such brief reviews is that the references are by no means a comprehensive listing of the literature in this area. The author apologizes in advance for any oversights, real or perceived.

GABAergic feedback inhibition

In the late 1970s, it was recognized that activation of baroreceptor afferents results in a sequence of excitation followed by inhibition in many NTS neurons. During extracellular recording of NTS neuronal responses to electrical activation of nerves containing baroreceptor afferent information (e.g., carotid sinus, vagus, aortic, superior laryngeal), this manifests as an initial excitatory response followed by an inhibition of discharge for up to several hundred milliseconds (Fig. 1A). During intracellular recording, electrical activation of cardiovascular afferent nerves evokes what has been assumed to be an analogous biphasic response pattern, and the components are designated as an excitatory postsynaptic potential (EPSP) and an inhibitory postsynaptic potential (IPSP) (Fig. 1B). The inhibition often observed after stimulation of baroreceptor afferent inputs can be graded as a function of stimulus intensity, generally occurs at the same or a slightly greater stimulus intensity than that necessary to evoke the excitatory component of the response, and temporally overlaps the excitatory component that limits the amplitude and duration of the excitatory response (Fig. 1B).

The inhibition often observed after stimulation of baroreceptor afferent inputs is primarily the result of activation of the type A receptors of γ-aminobutyric acid (GABA_A receptors). GABA_A receptors are postsynaptic, ionotropic, ligand-gated receptors, and their activation increases chloride conductance, which results in neuronal inhibition. The spontaneous discharge and discharge evoked by activation of baroreceptor afferent fibers in NTS neurons is markedly reduced or abolished following application of the GABA_A agonist muscimol (17). This inhibition of discharge appears to be equally dramatic in NTS neurons receiving monosynaptic and polysynaptic baroreceptor afferent inputs. Recent evidence indicates that GABA_B receptors might also contribute to the inhibition often observed after stimulation of baroreceptor afferent inputs (16). GABA_B receptors are metabotropic receptors that mediate both presynaptic inhibition (via reductions in calcium conductance that lead to reduced calcium-dependent exocytosis of transmitter from presynaptic nerve terminals) and postsynaptic inhibition (via increases in potassium conductance that lead to hyperpolarization of the postsynaptic membrane) (1). NTS neurons receiving polysynaptic baroreceptor afferent inputs are markedly inhibited by application of the GABA_B agonist baclofen, and this inhibition is mediated by both presynaptic and postsynaptic mechanisms (17). In contrast to neurons receiving polysynaptic inputs, NTS neurons receiving monosynaptic baroreceptor afferent inputs are comparatively insensitive to baclofen inhibition.

The contribution of presynaptic inhibition by GABA_B receptors to afferent-generated inhibition might be much greater than previously realized. Excitatory-inhibitory sequences are commonly observed during extracellular recordings from NTS neurons; 93% of NTS neurons receiving monosynaptic barore-
ceptor inputs and 95% of those receiving polysynaptic inputs respond to aortic nerve stimulation with excitation followed by inhibition. EPSP-IPSP sequences are not observed as frequently during intracellular recordings from NTS neurons; 33% of NTS neurons receiving monosynaptic baroreceptor inputs and 25% of those receiving polysynaptic inputs respond to aortic nerve stimulation with an EPSP-IPSP sequence. One possible interpretation of the disparity between extracellularly recorded excitation-inhibition and intracellularly recorded EPSP-IPSPs is that the extracellularly observed responses contain a component mediated by disfacilitation, which is the removal of an excitatory input and is typically not associated with a hyperpolarization of membrane potential. This disfacilitation would not be apparent in intracellular records unless the cell was spontaneously discharging action potentials.

Excitatory-inhibitory responses are also observed in the responses of some NTS neurons to natural activation of the arterial baroreceptors by increases in arterial pressure (Fig. 1C). The presence of an obvious excitatory-inhibitory response during an increase in arterial pressure is rare; only 12% of NTS neurons receiving monosynaptic baroreceptor inputs and 14% of neurons receiving polysynaptic inputs respond to an increase in arterial pressure with an obvious excitation-inhibition sequence (20). However, GABAergic inhibition is an important modulator of the NTS neuronal response to an increase in arterial pressure even in the absence of an obvious excitatory-inhibitory response during increases in arterial pressure. Suzuki et al. (12) found that application of the GABAa receptor antagonist bicuculline or the GABAb receptor antagonist phaclofen increased the peak discharge frequency response and prolonged the duration of the increase in discharge frequency of NTS neurons during an increase in arterial pressure (Fig. 1D). This further demonstrates that inhibition limits the excitatory response of NTS neurons to an increase in pressure. Many NTS neurons respond to increases in arterial pressure with only a transient increase in discharge frequency (20); therefore, GABAa and GABAb receptor-mediated inhibition is likely to be an important modulator of neuronal responses to changes in arterial pressure, even in those cells in which an obvious excitation-inhibition sequence is not observed.

In vivo studies have found that most, if not all, NTS neurons receive tonic GABAergic inputs; therefore, inhibition resulting from activation of baroreceptor afferent inputs might well be important in determining the spontaneous discharge of NTS neurons. Of course, tonic GABAergic inhibition might also arise from other inputs to the NTS, e.g., the paraventricular nucleus of the hypothalamus or the parabrachial nucleus. Tonic GABAergic inhibition might be responsible for the existence of “subthreshold neurons” within the NTS, neurons that do not respond to baroreceptor afferent activation unless their excitability is increased by application of excitatory amino acids (18). The resting membrane potential of subthreshold neurons was more hyperpolarized than the membrane potential of neurons that responded to baroreceptor afferent activation in the absence of exogenous application of excitatory amino acids; therefore, the subthreshold nature of the baroreceptor afferent input could be the result of tonic GABAergic inhibition or the relative absence of depolarizing synaptic and/or hormonal inputs. The observation that some second-order and higher-order NTS neurons receive subthreshold baroreceptor afferent inputs suggests the presence of a “reserve” population of neurons under certain conditions. A change from reserve to active status, either by a reduction in inhibition or an increase in excitatory input, will change the number of neurons involved in baroreflex circuits. This provides a mechanism for regulating reflex function independently of any alteration in the level of peripheral afferent input. The implication of this speculative mechanism is that modulation of baroreflex gain might be accomplished by not only modulating the discharge of NTS neurons but also by altering the number of neurons participating in the reflex.

**Temporal afferent interactions**

Numerous studies have demonstrated time- and frequency-dependent interactions between afferent inputs that could
modify the integration of baroreceptor afferent inputs within the NTS. Seller and Illert (10) first described frequency-dependent filtering of afferent inputs within the NTS, and subsequent studies indicate that this filtering is primarily the result of disfacilitation (5, 8). The characteristics of this frequency filtering are such that inputs stimulated at frequencies >10–20 Hz produce markedly reduced postsynaptic responses compared with lower-frequency (0.5–1.0 Hz) inputs (Fig. 2). Since the discharge of myelinated baroreceptor afferents can easily exceed 20 Hz during a single cardiac cycle, this high-frequency filtering has been proposed as a mechanism that limits the excitatory synaptic input to NTS neurons receiving baroreceptor afferent inputs. A recent finding of great interest is that there are differences in the degree of frequency filtering among NTS neurons. Lui et al. (5) reported that frequency-dependent inhibition is less in neurons receiving monosynaptic baroreceptor inputs compared with neurons receiving polysynaptic inputs. We have recently identified a subpopulation of NTS neurons receiving a monosynaptic baroreceptor afferent input that does not exhibit frequency-dependent inhibition during stimulus frequencies up to 50 Hz (K. Zhang and S. Mifflin, unpublished observations) in much the same manner as previously described for NTS neuronal responses to activation of laryngeal afferent inputs (Fig. 2). Therefore, the substrate exists for rapid transmission of the afferent input by some neurons receiving monosynaptic visceral afferent inputs. Such neurons may be involved in the rapid regulation of heart rate and/or sympathetic nerve activity.

A time-dependent form of inhibition has also been described in which prior activation of an afferent input can reduce NTS neuronal responsiveness to either the same or a different afferent input for 50–400 ms. This form of inhibition also appears to be due to disfacilitation or a reduction in the excitatory input to the cell (8). Studies examining the responses of NTS neurons to electrical stimulation of the aortic nerve in the rat, which appears to contain primarily baroreceptor afferent fibers, indicate that time-dependent inhibition is noticeably absent in most neurons receiving monosynaptic baroreceptor inputs (9). Previous studies using afferent nerves that relay more than one sensory modality (e.g., the carotid sinus or vagus nerves that relay mechanoreceptor and chemoreceptor information) and in vitro studies stimulating the fiber tract that carries all of these afferent inputs found time-dependent inhibition to be fairly ubiquitous among NTS neurons, and it is likely that this reflects interactions between different sensory modalities. For example, baroreceptor stimulation inhibits the responses of NTS neurons to activation of arterial chemoreceptors, presumably via presynaptic interactions, and this might explain the inhibitory interactions observed in the reflex responses to simultaneous activation of these two inputs (7).

What is the possible significance of these temporal interactions? It has been suggested that frequency-dependent inhibition within the NTS limits the inhibition of sympathetic outflow obtained during high-frequency aortic nerve stimulation (5). The peak discharge frequency responses to increases in arterial pressure are much lower in NTS neurons receiving polysynaptic afferent inputs compared with neurons receiving monosynaptic inputs (20), and the greater degree of frequency-dependent inhibition in the polysynaptic population might be responsible for this. Seller and Illert (10) suggested that the possible significance of these temporal interactions was to provide a "reserve capacity" to afferent integration. This reserve capacity was proposed to stabilize the discharge of NTS neurons in response to increases or decreases in the level of baroreceptor afferent input. Time- and frequency-dependent inhibitory interactions attenuate the increase in NTS neuronal discharge one...
might expect during an increase in arterial pressure by reducing the excitatory synaptic input to an NTS neuron. Conversely, the reduction in NTS neuronal discharge during a decrease in arterial pressure might not be as great as anticipated because time- and frequency-dependent interactions are lessened, allowing remaining excitatory inputs to be more fully expressed. Concerning this, we have recently described (20) that fairly dramatic reductions in pressure produce very modest reductions in the spontaneous discharge of NTS neurons. This spontaneous discharge is due to synaptic excitation because it can be abolished by application of non-N-methyl-D-aspartate receptor antagonists (18). The reserve capacity provided by temporal interactions might minimize the effects of reducing the intensity of a particular excitatory afferent input.

**Baroreceptor afferent integration in hypertension**

Studies of baroreflex function in hypertension have yielded conflicting results, some studies indicating normal, reduced, or even enhanced reflex gain in chronically hypertensive animals and humans. This variability is probably related to a variety of factors, such as the model of hypertension, the duration and severity of hypertension, and the species studied. The general consensus is that chronic hypertension is associated with either normal or reduced baroreflex function.

As might be expected given the previous discussion of GABA_B receptor-mediated inhibition of NTS neurons, the microinjection of the GABA_B agonist baclofen into the NTS results in baroreflex inhibition and an increase in arterial pressure. In several models of hypertension (SHR, DOCA-salt, and renal wrap hypertensive rats) this GABA_B receptor-induced pressor response is greater than the pressor response evoked by similar injections in normotensive animals, suggesting an upregulation of GABA_B receptor function in the NTS of hypertensive animals (2, 13). In chronic hypertensive animals, NTS neurons receiving monosynaptic baroreceptor afferent inputs exhibit enhanced inhibitory responses to application of baclofen (J. Zhang and S. Mifflin, unpublished observations); recall that in control animals these neurons are fairly insensitive to baclofen inhibition (17). Chronic hypertension is associated with increased expression of GABA_B receptor mRNA within the NTS (2). A recent study suggested that the increased GABA_B inhibition in chronically hypertensive animals is the result of an increase in both the presynaptic and postsynaptic components of the baclofen-induced pressor response (15). Increased baroreceptor afferent input to the NTS in hypertension (see below) is a likely explanation for the increase in the presynaptic component of the baclofen response. It also appears that some factor or factors associated with chronic hypertension, for example increased excitatory or inhibitory synaptic input and/or a circulating hormone, leads to increased expression of the postsynaptic GABA_B receptor and enhanced GABA_B receptor function within the NTS.

The functional significance of the enhanced inhibition that accompanies chronic hypertension may become apparent if we consider the overall level of excitatory baroreceptor afferent input to the central nervous system in hypertension. The landmark studies of Kreiger’s group (4) established the concept that baroreceptor afferents undergo a “complete resetting” of their discharge responses to increases in pressure during hypertension, so that the central nervous system receives a “normal” level of excitatory afferent input despite the increased arterial pressure. What has not been appreciated is that, although the responses of individual, presumably myelinated (due to their regular and high-frequency discharge) baroreceptor afferents may be “normal,” the absolute number of baroreceptor afferents discharging at a given pressure is increased in chronic hypertension. Jones and Thoren (3) demonstrated this for chronic renal hypertensive rabbits (Fig. 3). In normotensive animals, ~91% of myelinated and ~28% of unmyelinated baroreceptor afferent fibers discharge at the resting levels of arterial pressure. In chronically hypertensive rabbits, ~100% of myelinated and ~78% of unmyelinated baroreceptor afferent fibers discharge at the resting levels of arterial pressure. This indicates a dramatic increase in the amount of tonic excitatory baroreceptor afferent input to the central nervous system in chronic hypertensive animals. This increase in baroreceptor afferent excitatory input in early hypertension is illustrated in the baroreflex curves schematized in Fig. 4A as movement along the line designated (1). The postulated effects of an acute increase in baroreceptor excitatory inputs to an NTS neuron is schematized in Fig. 4B as movement along the line designated (1).

The frequency- and time-dependent interactions discussed above are possible mechanisms that might limit the effects of this increase in excitatory input to the central nervous system during hypertension. However, Scheuer et al. (9) reported that time-dependent inhibition in NTS neurons receiving monosynaptic baroreceptor afferent inputs was less in animals with elevated levels of arterial pressure. As previously discussed, the degree and prevalence of frequency-dependent inhibition is not dramatic in many NTS neurons receiving a monosynaptic baroreceptor input (5). Our preliminary results suggest that frequency-dependent inhibition in NTS neurons receiving mono-

**FIGURE 3.** Baroreceptor discharge in chronically hypertensive rabbits. Percentage of myelinated and unmyelinated afferent fibers active at each stepwise increment in arterial pressure in normotensive (top) and chronic hypertensive (bottom) animals are shown. The arrows on the abscissa mark the resting level of arterial pressure in each group. Reproduced from Ref. 3 with permission.
afferent fibers in hypertensive animals results in blunted reflex processing of the baroreceptor signals by the NTS neurons. The normal resting level of arterial pressure is defined as the operating point (green circle) on the normotensive curve. If the system remained at the point indicated by the blue circle in Fig. 4A, the baroreflex would not be capable of responding to increases in arterial pressure very well. Since most reflex studies in hypertensive animals indicate that baroreflex curves are shifted to the right, as schematized in Fig. 4A, most investigators have concluded that adaptations occur within the baroreflex arc in chronic hypertension. It is proposed that enhanced GABA_β-mediated inhibition counteracts or offsets the increased excitatory baroreceptor afferent input to NTS neurons in hypertension, and this is illustrated as movement along line 2 in Fig. 4B. This results in a normalization of NTS neuronal discharge so that the neuron is again capable of responding to increases or decreases in arterial pressure. In support of this, the majority of NTS neurons receiving baroreceptor inputs in hypertensive rats exhibit spontaneous discharge frequencies and responses to baroreceptor afferent stimulation that are no different from the responses observed in normotensive rats (19). Enhanced inhibition in hypertensive animals could also be responsible for the phenomenon of central resetting, in which electrical stimulation of baroreceptor afferent fibers in hypertensive animals results in blunted reflex responses (19). The proposed model can also account for situations in which baroreflex regulation of end-organ responses is reduced, because situations in which the match between the increase in excitatory baroreceptor afferent input and the increase in GABA_β receptor-mediated inhibition is not perfect. It is proposed that these changes at the level of the NTS restore the regulatory capabilities of the baroreflex, illustrated as movement along line 2 in Fig. 4A. This model suggests that baroreflex regulation of the absolute resting level of arterial pressure is sacrificed to maintain the ability of the baroreflex to minimize deviations in blood pressure from the resting level.

To conclude, the processes that mediate baroreceptor afferent integration within the NTS include an impressive array of inhibitory mechanisms. Inhibitory processes will modify the signal that is relayed to subsequent central nuclei in the baroreflex arc. It now appears that an additional layer of complexity must be considered because inhibitory modulatory mechanisms may themselves be modified. The concept that the characteristics of neurons and networks change following chronic alterations in the level of synaptic inputs to maintain an optimal or preferred range of discharge frequency was recently reviewed by Turrigiano (14). Termed “homeostatic plasticity,” these changes in neuronal discharge characteristics are proposed to reestablish some degree of regulatory capability to the network. To fully appreciate how homeostatic plasticity within the NTS might impact overall baroreflex function in hypertension, it is useful to examine what is happening in central nuclei.
subsequent to the NTS integration of the baroreceptor afferent input. One such site, the rostral ventrolateral medulla, contains neurons that are inhibited by activation of baroreceptor afferent fibers and are considered a major determinant of sympathetic vasconstrictor tone. The spontaneous discharge and baroreceptor inhibition of neurons in the rostral ventrolateral medulla is normal in spontaneously hypertensive rats (11). Therefore, the increased baroreceptor afferent input to the central nervous system in hypertension (Fig. 3) appears to be normalized before the rostral ventrolateral medulla, possibly by the changes observed in the NTS. It will be interesting to determine if adaptive changes accompany other conditions in which the level of peripheral afferent inputs to the central nervous system are chronically altered (e.g., chronic hypoxia, heart failure, bulimia, sleep apnea, or esophageal reflux).

I thank Drs. J. R. Haywood and G. M. Toney for their comments on a draft version of this manuscript.

I gratefully acknowledge the support of National Heart, Lung, and Blood Institute Grants HL-41894 and HL-56637.

References

16. Wang Y and Jordan D. GABA\textsubscript{A} and GABA\textsubscript{B} receptors are involved in vagal afferent inhibitory input to neurones in the nucleus tractus solitarius. J Physiol (Lond) 504: 197P–198P, 1997.