Plasticity of GABAergic Mechanisms Within the Nucleus of the Solitary Tract in Hypertension

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Adaptive changes have long been recognized to occur in the heart and vasculature in response to chronic hypertension. What might be less well-appreciated is the fact that chronically increased blood pressure is also associated with adaptive changes in neurons within the central nervous system (CNS). Changes in the properties of ligand-gated and voltage-gated channels have been described in a variety of neurons in a variety of central nuclei and in a variety of models of hypertension.

Gamma-aminobutyric acid (GABA) is the main inhibitory neurotransmitter in virtually every region in the adult brain. Microinjections of GABA and GABA receptor subtype-selective agonists and antagonists have been performed within various cardiovascular-related regions of the CNS. In every cardiovascular-related region of the CNS tested, activation of GABA receptors alters cardiovascular function. This is likely attributable to the ubiquitous role of GABA within the CNS. GABAergic inhibition can be mediated by activation of receptors located in presynaptic and postsynaptic loci. Two major subtypes of the GABA receptor exist: the GABA<sub>A</sub> receptor is a pentameric, chloride ionophore that primarily mediates postsynaptic inhibition, whereas the GABA<sub>B</sub> receptor is a G-protein-coupled receptor that can induce reductions in calcium conductance to mediate presynaptic inhibition and increases in potassium conductance to mediate postsynaptic inhibition.

GABAergic inhibition of central neurons can result in pressor or depressor responses depending on the central site being examined. Pressor responses are often assumed to be the result of GABAergic inhibition of neurons that reduce sympathetic discharge. Depending on the specific area being studied, GABA injections into the CNS can also alter vagal cardiac function and levels of vasoactive hormones such as vasopressin and angiotensin, in addition to changes in sympathetic outflow.

In addition to microinjection studies, in vivo and in vitro electrophysiological analyses of functionally identified neurons in cardiovascular-related CNS areas are useful in the analysis of mechanisms that underlie alterations in GABAergic neurotransmission in hypertensive animals. Because of space constraints this review selectively summarizes changes in GABAergic transmission in hypertensive rats that have recently been described in the nucleus of the solitary tract (NTS), the first integrative site for baroreceptor afferent inputs within the CNS.

Nucleus of the Solitary Tract and Hypertension

Within caudal regions of the NTS, microinjection of GABA<sub>A</sub> or GABA<sub>B</sub> receptor agonists increase arterial pressure, presumably attributable to GABAergic inhibition of NTS neurons receiving arterial baroreceptor afferent inputs. Furthermore, microinjection of GABA<sub>A</sub> or GABA<sub>B</sub> receptor antagonists lower arterial pressure, indicating that GABAergic inhibition via both receptor subtypes is a tonically active process within the NTS. The pressor and sympatho-excitatory responses to activation of GABA<sub>B</sub> receptors are enhanced in spontaneously hypertensive rats (SHR), DOCA-salt, and renal-wrap hypertensive rats. In contrast, pressor and sympatho-excitatory responses to activation of GABA<sub>A</sub> receptors are enhanced in spontaneously hypertensive rats (SHR), DOCA-salt, and renal-wrap hypertensive rats. These microinjection studies strongly suggest hypertension-induced alterations in GABAergic mechanisms in the NTS. However, because of inherent limitations of the microinjection technique, they provide little insight into the specific changes that occur in individual NTS neurons. Electrophysiological analyses of NTS neurons receiving arterial baroreceptor inputs provide insights into hypertension-induced neural plasticity of GABAergic mechanisms in the NTS.

The responses of individual NTS neurons that receive arterial baroreceptor afferent inputs to exogenous application of GABA receptor selective agonists have been examined in normotensive and in renal-wrap hypertensive rats. An in vivo study of NTS neurons receiving baroreceptor inputs, and therefore presumed to be sympathetic-inhibitory in function, found that the ability of the GABA<sub>A</sub> receptor agonist muscimol to inhibit aortic nerve (baroreceptor) evoked discharge was reduced after 1 and 4 weeks of hypertension. Conversely, the ability of the GABA<sub>B</sub> receptor agonist baclofen to inhibit aortic nerve evoked discharge was enhanced after 1 and 4 weeks of hypertension. The changes in sensitivity to

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activation of GABA_A and GABA_B receptors were found to occur in NTS neurons that received rapidly conducting baroreceptor afferent inputs and in NTS neurons that received slowly conducting baroreceptor afferent inputs. The results demonstrate that early in hypertension the sensitivity of NTS neurons to activation of GABA receptors is altered and that these changes are maintained for at least 4 weeks. Directionally opposite changes in sensitivity occur in response to GABA_A (reduced inhibition) and GABA_B (enhanced inhibition) receptor selective agonists.

In vitro electrophysiological analyses provide additional insights to those obtained in vivo, particularly if one attempts to obtain some level of identification of neuronal function in the in vitro setting to demonstrate potential functional significance. Anatomic labeling techniques have been used to identify NTS neurons receiving aortic nerve (baroreceptor) inputs in in vitro preparations so that this specific class of neuron can be studied. In labeled neurons isolated from the NTS of renal-wrap hypertensive rats, changes in the postsynaptic responses to activation of GABA_A and GABA_B receptors mirrored the changes observed in vivo. The midpoint of the dose–response curve (EC50) for peak GABA_A currents was significantly greater in neurons from hypertensive compared with normotensive rats. The time constant for desensitization of GABA_A evoked currents was the same in neurons from hypertensive and normotensive rats, indicating that the reduced sensitivity was not associated with a change in desensitization of GABA_A evoked responses. The alterations in GABA_A receptor-evoked currents are consistent with the in vivo observations and indicate that NTS neurons receiving arterial baroreceptor inputs are less sensitive to GABA_A receptor inhibition.

GABA_B receptors can mediate both presynaptic and postsynaptic inhibition. In vitro analyses of GABA_B-evoked responses were performed using a brain slice preparation so that both the presynaptic and postsynaptic components of GABA_B-mediated inhibition could be analyzed in NTS neurons receiving arterial baroreceptor afferent inputs. GABA_B-evoked postsynaptic responses were consistent with the in vivo observations. The EC50 of the postsynaptic outward potassium current induced by application of the GABA_B agonist baclofen was significantly less in NTS neurons recorded in brain slices from hypertensive compared to normotensive rats, suggesting an enhanced postsynaptic response to activation of GABA_B receptors in second-order baroreceptor neurons in the NTS in renal-wrap hypertensive rats.

More recent studies in our laboratory indicate alterations also occur in the presynaptic GABA_B receptor that could influence glutamate and GABA release in hypertension. Recordings were obtained from NTS neurons in a brain slice using pipettes filled with cesium to block potassium currents so that presynaptic GABA_B effects could be observed in the absence of postsynaptic GABA_B-evoked outward current. Baclofen reduced the amplitude of tractus-evoked excitatory postsynaptic currents in the absence of a direct effect on the postsynaptic membrane, suggesting that reduced excitatory postsynaptic currents amplitude was the result of GABA_B receptor-mediated presynaptic inhibition. The EC50 of the GABA_B receptor-mediated presynaptic inhibition of evoked excitatory postsynaptic currents amplitude was significantly reduced in hypertensive compared to normotensive rats. The results suggest that in renal-wrap hypertensive rats, baclofen evokes an enhanced presynaptic inhibition of glutamate release in second-order baroreceptor neurons.

**Nucleus of the Solitary Tract and Exercise**

During exercise, the arterial baroreflex is reset to operate at higher pressures in humans. GABAergic mechanisms in the NTS have also been shown to be involved in exercise-induced resetting of the baroreflex in the rat. Somatosensory afferent fibers originating from skeletal muscle are activated during exercise and release substance P in the NTS, which directly activates NTS GABAergic neurons. The GABAergic neurons then inhibit NTS neurons receiving baroreceptor inputs to reset the reflex function curve. The model is similar to that proposed for reflex resetting in hypertension in which the resetting moves the set-point to the middle (linear) range of the reflex function curve.

In relation to hypertension, a single bout of mild to moderate exercise can lead to a prolonged, postexercise decrease in blood pressure in hypertensive subjects. Studies in the SHR have shown that during this “postexercise hypotension” the release of GABA is reduced within the NTS; therefore, the inhibition of NTS neurons by somatic afferents is reduced, contributing to a reduction in blood pressure. The reduced release of GABA was shown to be associated with internalization of the substance P (NK-1) receptor so that somatic afferent activation during exercise produces less excitation of the GABAergic neurons. It is not known if the reduced substance P-mediated excitation of GABAergic neurons is a result of the hypertension.

**It Is Not All About GABA**

Neuronal adaptations to hypertension within the NTS are not restricted to alterations in GABAergic mechanisms. Anatomic studies report alterations in excitatory amino acid AMPA receptor subunits within the NTS in hypertension. Hypertension also induces alterations in voltage-gated ion channels within the NTS. In renal-wrap hypertensive rats, the transient outward potassium current (Ih), a K current contributing to neuronal excitability, is reduced in NTS neurons receiving arterial baroreceptor inputs, whereas delayed rectifier potassium currents are normal. Delayed excitation, one current-clamp manifestation of Ih, has also been reported in NTS neurons in SHR. The recent finding that many NTS neurons that exhibit Ih project to the paraventricular nucleus of the hypothalamus suggests that this alteration could influence the baroreceptor signal that is relayed to the hypothalamus and subsequently sympathetic outflow.

Hypertension also induces enhanced current flow through high-voltage-activated, but not low-voltage-activated, Ca2+ channels in NTS neurons that receive baroreceptor afferent inputs. There is a link between enhanced calcium influx and reduced GABA_A receptor function. Increased Ca2+ influx, as a result of synaptic depolarization or depolarizing voltage pulses, reduces the neuronal response to activation of GABA_A receptors.

These findings emphasize that neurons exhibit a number of adaptive changes in hypertension. There are no doubt numer-
ous other changes in these neurons and in other regions of the CNS that have yet to be described. Present efforts to provide a comprehensive model that explain alterations in neural regulation of cardiovascular function in hypertension are limited by the incomplete state of our current knowledge regarding the full extent of the adaptations; that is, what changes and where?

**What Alters GABA Receptor Function in Hypertension?**

What might initiate neuronal adaptations to chronic hypertension? The in vitro findings have an important implication in that whatever alters the sensitivity of second-order NTS neurons to the activation of GABA_A and GABA_B receptors, the altered sensitivity persists at least for several hours in the absence of the hypertension because of the nature of in vitro preparation. Increased levels of GABA_B mRNA have been reported in the NTS of both renal-wrap hypertensive and SHR, so enhanced GABA_B responses in hypertension might be attributable to increased receptor levels if the increased message is translated into increased receptor protein.

The pressure sensitivity of arterial baroreceptors “resets” in hypertension so that the threshold pressure necessary to evoke discharge is elevated. Suprathreshold sensitivity may, or may not, be normal; however, it is important to realize that the absolute number of baroreceptor afferents discharging at a given pressure is increased in chronic hypertension. In normotensive rabbits, ~91% of myelinated and 28% of unmyelinated baroreceptor afferent fibers are discharging at the resting levels of arterial pressure. In chronically hypertensive rabbits, ~100% of myelinated and 78% of unmyelinated baroreceptor afferent fibers are discharging at the resting hypertensive level of arterial pressure. This suggests a large increase in tonic excitatory baroreceptor afferent input to the NTS in chronic hypertensive animals, primarily as a result of recruitment of unmyelinated afferent fibers.

Therefore, alterations in GABA receptor function could be the result of an increased baroreceptor afferent input to the NTS and the subsequent effect(s) of increased discharge or increased exposure to neurotransmitters. We have found reduced levels of message for the GABA_A-α1 subunit in the NTS of renal-wrap hypertensive rats (Figure 1). This suggests that the reduced GABA_A receptor responses observed in vivo and in vitro may be the result of reduced levels of GABA_A receptor. This reduction is abolished by sectioning the carotid sinus and aortic depressor nerves to eliminate baroreceptor afferent inputs to the NTS before the onset of hypertension, suggesting that the reduced expression of the NTS GABA_A-α1 subunit in hypertension is dependent on baroreceptor afferent inputs to the NTS.

If excitatory drive to the NTS is increased in hypertension, then one would predict that these neurons would exhibit an increased discharge frequency in hypertensive animals. However, the discharge frequency of NTS neurons receiving arterial baroreceptor inputs is not different in renal-wrap hypertensive rats. It has been proposed that increased GABA_B receptor-mediated inhibition may limit the excitatory drive to NTS neurons in hypertension.

Factors that regulate GABA_B receptor function are less well-studied than those that regulate GABA_A receptor function. The availability of surface GABA_B receptors is regulated by glutamate in hippocampal and cortical neurons; however, the results are not consistent. Activation of GABA_B receptors with baclofen did not influence receptor endocytosis in these neurons, but it did increase receptor degradation through mechanisms that were independent of endocytosis. Increased calcium influx through voltage-gated calcium channels has been described in the NTS of hypertensive rats, and changes in intracellular calcium could initiate alterations in gene expression that influence any of the numerous mechanisms shown to alter GABA_A and GABA_B receptor function (eg, receptor expression, subunit composition, trafficking, phosphorylation status). Transcription factors that could mediate neuronal adaptations in hypertension include the immediate early gene product c-Fos and the intermediate factor FosB (Cunningham and Mifflin, unpublished observations).

Other factors to consider as initiators of neuronal adaptation in hypertension are changes in tissue or systemic levels of hormones induced by chronic hypertension. These neurochemicals may also cause alterations in neural structures not immediately involved in baroreflex. Many of the changes reported in NTS neurons from hypertensive rats (alterations in K^- and Ca^{2+} channel conductances) are similar to the acute effects of angiotensin II (Ang II) acting on AT1 receptors on brainstem neurons. In addition to direct effects on NTS neurons receiving arterial baroreceptor inputs, recent work suggests that Ang II can also activate endothelial nitric oxide synthase, and the resulting release of nitric oxide stimulates GABA release and inhibition of NTS neurons receiving arterial baroreceptor inputs.
Ang II may also play a role in initiating hypertension-induced changes in GABA<sub>B</sub> receptor function because recent in vitro work found that application of Ang II to NTS neuronal cultures induce a 2-fold increase in GABA<sub>B</sub> receptor expression.<sup>40</sup> Treatment of the NTS neuronal cultures with baclofen decreased neuronal discharge frequency by a greater amount in cultures pretreated with Ang II, indicating that chronic Ang II treatment significantly enhanced the neuronal response to GABA<sub>B</sub> receptor activation. Ang II had no effect on the inhibitory action of the GABA<sub>A</sub> receptor agonist muscimol, suggesting the actions of Ang II were selective for the GABA<sub>B</sub> receptor. In whole animal studies, intracerebroventricular infusion of Ang II was associated with an elevation of GABA<sub>B</sub> receptor mRNA and protein levels in the NTS; however, there was an Ang II-induced increase in mean arterial pressure (MAP) that could contribute to the observation. These results indicate that Ang II stimulates GABA<sub>B</sub> receptor expression in NTS neurons. Because the renal-wrap model of hypertension is Ang II-dependent,<sup>41</sup> Ang II could mediate changes in GABA<sub>B</sub> receptor function reported in the NTS of renal-wrap hypertensive rats.<sup>8,15</sup> It would be interesting to examine GABA-evoked responses in renal-wrap hypertensive rats after prolonged blockade of Ang II receptors within the NTS. In addition to an increase in receptor protein, Ang II could also increase GABA<sub>B</sub> receptor function via common G-protein-coupled receptor signal transduction pathways activated by Ang II and GABA<sub>B</sub> receptors.

### Specificity and Selectivity

To this point the question has been framed, “What happens to neurons receiving a baroreceptor afferent input during chronic hypertension?” A question that arises from these observations is to what extent are neuronal adaptations to hypertension within the NTS restricted to neurons receiving arterial baroreceptor afferent inputs?

The in vivo study cited previously<sup>42</sup> examined responses of NTS neurons that did not receive aortic nerve inputs to iontophoretic application of GABA<sub>A</sub> and GABA<sub>B</sub> receptor agonists. There was no change in the inhibition of spontaneous discharge induced by activation of GABA<sub>A</sub> receptors, whereas inhibition induced by activation of GABA<sub>B</sub> receptors was enhanced as observed in neurons receiving aortic nerve inputs. The in vitro analysis of NTS neuronal responses to activation of GABA<sub>A</sub> receptors found no difference in the dose–response relationship of neurons receiving aortic nerve inputs and neurons not receiving aortic nerve inputs, although the sample size was small.<sup>14</sup> Similarly, changes in transient outward potassium currents<sup>24</sup> and high-threshold voltage-gated calcium channels<sup>27</sup> did not differ comparing NTS neurons that received aortic nerve inputs to those that did not receive aortic nerve inputs.

It is possible that at least some portion of the nonaortic nerve-activated population of NTS neurons in these studies receive inputs from other arterial or cardiac/thoracic baroreceptors. The model hypothesized to explain alterations in NTS neuronal responses in hypertension proposed that increased baroreceptor afferent input to the NTS was the primary initiator of the neuronal adaptations.<sup>19</sup> The finding that GABA<sub>A-<small>α1</small></sub> receptor subunit mRNA levels are reduced in hypertensive rats and that this reduction can be abolished by section of baroreceptor afferent nerves is consistent with this hypothesis. However, removal of baroreceptor afferent inputs is likely to alter numerous other factors that could influence the neurons, e.g., circulating or tissue levels of hormones, hind brain blood flow, glucose, or oxygen availability. The fact that a reduction in receptor subunit levels is observed is surprising given the relatively small number of cells within the NTS that receive an arterial baroreceptor input and suggests that alterations in GABA-gated receptors and voltage-gated ion channels occur in a larger population of NTS neurons than those that receive solely arterial baroreceptor afferent inputs.

If neuronal adaptations occur in a larger population of NTS neurons, one might predict that other reflex or integrative functions of NTS neurons not involved in baroreflexes would be altered in hypertensive animals. Alterations in cardiopulmonary mechanoreflexes<sup>43,44</sup> and arterial chemoreflexes<sup>45</sup> have been reported in hypertensive rats.

### Significance of Alterations in Central GABAergic Mechanisms in Hypertension

At present it is difficult to estimate the contribution of the hypertension induced changes in GABAergic mechanisms in the hind brain to cardiovascular regulation in hypertension. In the context of cardiovascular regulation and hypertension, altered GABAergic neurotransmission is only one of a myriad of factors in the overall adaptive response. These and other changes are likely to occur in other central areas and have yet to be fully characterized. For example, a “tonic GABA<sub>A</sub> current” has been identified in paraventricular neurons that project to the rostral ventrolateral medulla.<sup>46</sup> The extent to which this current, if present, is altered in the NTS or any other brain region in hypertension has yet to be examined.

Some insight can be gleaned by looking at the output side of the CNS. The tonic discharge frequency of putative sympatho-excitatory neurons within the rostral ventrolateral medulla is normal in the SHR and baroreflex inhibition of this discharge appears normal, albeit shifted toward higher pressures.<sup>47</sup> Using the immediate early gene product c-Fos as an indicator of neuronal activation, the number of neurons exhibiting c-Fos is elevated in the NTS, caudal ventrolateral medulla, rostral ventrolateral medulla, and paraventricular nucleus in renal-wrap hypertensive rats.<sup>36</sup>

A model whereby increased GABA<sub>B</sub> receptor function offsets, to some extent, increased excitatory baroreceptor input to NTS neurons has been proposed.<sup>19</sup> The increased number of active neurons in the NTS of hypertensive rats described in the c-Fos study<sup>36</sup> suggests that any reduction in excitatory synaptic input mediated by increased GABA<sub>B</sub> receptor presynaptic inhibition is not sufficient to prevent recruitment of additional neurons. However, it appears to be sufficient to normalize NTS discharge that remains normal, ensuring that discharge remains at a level at which the neuron can still respond to increases or decreases in MAP. If increased GABA release in the paraventricular nucleus in
renal-wrap hypertensive rats\textsuperscript{48} is dependent on the NTS, then the model predicts that it is the result of an increased number of active NTS neurons and not an increased discharge in any given neuron. This model is also consistent with the finding of normal baroreflex inhibition of rostral ventrolateral medulla neurons in SHR.\textsuperscript{47}

A study by Haywood et al\textsuperscript{49} emphasizes the important role that the baroreflexes play in normotension and hypertension and may provide some insight into the significance of neuronal adaptations in NTS neurons receiving arterial baroreceptor inputs. In renal-wrap hypertensive rats, MAP variability is increased by $\approx 50\%$ (Figure 2). This indicates a diminution in baroreflex buffering capability, although contributions from the neuro-effector junction cannot be excluded. Sino-aortic denervation, to eliminate baroreceptor inputs to the CNS, in normotensive rats also increased MAP variability by $50\%$. However, in sino-aortic denervation renal-wrap hypertensive rats, MAP variability was nearly double that in sino-aortic denervation normotensive rats. Therefore, the contribution of the baroreflexes to the mechanisms that serve to minimize MAP variability is actually much greater in hypertensive compared to normotensive animals. Increased MAP variability has been associated with increased risk of cardiovascular mortality because of myocardial infarction, stroke, end-organ damage such as for stroke, and end-organ (heart, kidney, blood vessel) damage.\textsuperscript{50–58} There is an ongoing debate of the role of the arterial baroreflexes in the determination of the absolute level of MAP in normotension and hypertension. Regardless, the role of the arterial baroreflexes in the determination of the stability of MAP is not in dispute and it may well be that the ability to maintain some degree of baroreflex buffering that is of equal clinical significance in hypertension.

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References


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