Autonomic-Immune-Vascular Interaction
An Emerging Concept for Neurogenic Hypertension

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Hypertension is the single most important risk factor for cardiovascular disease and, thus, remains a global public health challenge. Significant progress has been made during the last several decades in the treatment of hypertension through the use of inhibitors of the renin-angiotensin (Ang) system, with either Ang-converting enzyme inhibitors or Ang receptor type 1 blockers, diuretics, α-adrenoreceptor antagonists, and calcium channel blockers. Despite these advances, it has been extremely difficult to manage hypertension in ≈40% of hypertensive patients.1 A majority of these unresponsive patients exhibit increased sympathetic drive and display neurogenic components.2–4 The role of elevated sympathetic nervous system in drug-resistant hypertension is corroborated by the recent successes in its treatment by renal sympathetic denervation in humans.5,5,6 These observations have led to the realization that elucidation and understanding of cellular, molecular, and physiological mechanisms involved in the development and establishment of neurogenic hypertension are pivotal in advancing new therapeutic strategies that would be effective in a majority of patients. The objectives of this article are to summarize recent advances in this field, underscoring the importance of coordinated neural-peripheral signaling in neutral control of blood pressure (BP) and contribution of inflammatory molecules, and to introduce novel and emerging concepts in the pathogenesis of neurogenic hypertension. It is advised to consult other reviewers for details on fundamental studies related to neural pathways, the renin-Ang system, and involvement of other hormonal systems in neurogenic hypertension.7–13

Neurogenic Hypertension
An alteration in neural cardiovascular (vagal/sympathetic) control mechanism is the principal characteristic of neurogenic hypertension. Studies demonstrating a significant enhancement of the vasomotor and cardiac sympathetic drive, as well as a reduction in the parasympathetic drive in borderline hypertensive patients, support this premise.14–16 In addition, hypertensive patients show a greater plasma norepinephrine level, and greater norepinephrine spillover in both the peripheral circulation and blood draining from the brain, as well as raised sympathetic postganglionic activity targeting the skeletal muscle vascular bed.17–20 Animal models corroborate the human data. Recent evidence shows that even the monogenic forms of hypertension, such as the apparent mineralocorticoid excess, which greatly depends on salt-absorptive mechanisms of the kidney, have also been attributed to an elevated sympathetic drive, indicating a very close association between renal dysfunction and sympathetic overdrive.21,22 Equally, the Goldblatt model partially depends on signaling within the rostral ventrolateral medulla for the maintenance of hypertension.23

Evidence from the spontaneously hypertensive rat (SHR), a well-established animal model for human hypertension, supports the idea that sympathetic overdrive precedes hypertension.24 Therefore, molecular and neuronal changes at the level of the brain stem and hypothalamus may contribute to neurogenic hypertension. Our studies have demonstrated that elevated renin-Ang system in the brain stem and hypothalamus of juvenile SHRs affects the regulation of arterial pressure and baroreceptor reflex gain in the adult SHRs.25–28 Moreover, enhanced central respiratory-sympathetic coupling in rat models, which are already present at a prehypertensive age, contributes to raised vasomotor tone.13,24,29,30 Thus, sympathetic activation appears to not only initiate hypertension but also to maintain it. Whether elevated sympathetic activity is causal to the initiation of hypertension remains equivocal, but it certainly occurs early in the condition.

Inflammation, the Blood-Brain Barrier, and Hypertension
Overwhelming evidence, both clinical and experimental, demonstrates that hypertension is associated with circulating levels of inflammation markers. In particular, there is strong support of the concept that the vascular inflammatory process plays an important role in the pathophysiology of hypertension (Figure 1). Cross-sectional and prospective studies have demonstrated that circulating levels of inflammatory molecules, such as C-reactive protein, tumor necrosis factor α-α (TNF-α), interleukin (IL) 6, monocyte chemoattractant protein 1, and adhesion molecules, such as P-selectin and
intercellular adhesion molecule 1, are increased in patients with primary hypertension.31–35 The Women’s Health Study indicated that C-reactive protein levels predicted the development of hypertension, an association that was independent of BP. Finally, inhibitors of the renin-Ang system, which are effective antihypertensive in some patients, are shown to decrease C-reactive protein, IL-6, TNF-α levels, and inflammatory status.31–35 Animal studies have supported these clinical association studies and have been instrumental in furthering our understanding of the involvement of inflammatory mechanisms in vascular dysfunction and the hypertensive condition. For example, total leukocyte counts in young, mature, and old SHRs were 50% to 100% above the controls with the number of monocytes, activated monocytes, and the lymphocyte counts significantly elevated.36 Leukocyte-endothelial interactions were also reported to be altered in the SHRs.36 Moreover, the elevated BP, above normal leukocyte counts, and elevated number of activated neutrophils in SHRs were all suppressed after adrenalectomy, indicating a relationship between altered leukocyte adhesiveness and adrenal corticosteroids in SHRs.36 Blood transfusions from SHRs to Wistar-Kyoto rats increased BP because of the more sticky nature of the SHR leukocytes.37 The “stickier” nature of leukocytes has been attributed to the elevated glucocorticoid levels in the SHR, which induces leukocyte pseudopod projections and results in slower cell passage through capillaries and elevated hemodynamic resistance in the SHR.38 Furthermore, elevated Ang II promotes leukocyte-endothelial interaction, thereby contributing to vascular inflammation.38 T cells play an important role in Ang II infusion, desoxycorticosterone acetate-salt-induced hypertension, and associated vascular and renal dysfunctions.39–41 The T-cell modulating agent, mycophenolate mofetil, prevents hypertension in animal models.41 The SHR exhibits increased levels of activated monocytes, of which levels are significantly decreased by reducing BP.46 Vascular inflammation in this rat model of hypertension was increased along with increases in expression of inflammatory cytokines IL-1β, IL-6, and TNF-α. The Ang II type 1 (AT1) receptor blocker, candesartan, produced antihypertensive effects and decreases in inflammatory cytokines.42 Similarly, an association between inflammation and hypertension has been demonstrated in aldosterone models. For example, circulating levels of inflammatory markers are increased by aldosterone infusion in humans and patients with primary hyperaldosteronism, which is associated with vascular dysfunction, car-
diabetic fibrosis, and increased risk of myocardial infarction and stroke.31 Chronic treatment of hypertensive rats with mineralocorticoid receptor antagonist, which attenuates BP, decreases circulating levels of certain inflammatory cytokines and renal and cardiac expression of inflammatory mediators, such as IL-1, IL-6, and nuclear factor κB.31

The association between inflammation and inflammatory cytokines and chemokines and hypertension is evident from the above discussion. However, the concept of the involvement of the brain in inflammatory mechanisms is still evolving. For example, inflammatory cytokines such as TNF-α and IL-1β induce cyclooxygenase 2 activity in perivascular macrophages of the blood-brain barrier (BBB) and generate prostaglandin E2, which enters the brain and stimulates paraventricular nucleus (PVN) neurons regulating adrenocorticotropic hormone release and sympathetic drive.43,44 In addition, expression of junctional adhesion molecule 1 is significantly increased in the nucleus of the solitary tract (NTS) of SHRs relative to Wistar-Kyoto rats.45 This increased expression of junctional adhesion molecule 1 induces both hypertension (in normotensive rats) and leukocytes accumulation in the NTS microvasculature and may lead to enhancement of cell transmigration across the tight junctions, activation of platelet aggregation, and increased production of cytokines, thereby contributing to the inflammatory status of the NTS, which may then directly or indirectly regulate NTS neuronal activity and baroreceptor functions. Ando et al42 have also demonstrated that inflammation in the SHR is not restricted to the peripheral vessels, but it is also extended to the cerebral vasculature. Treatment of SHRs with Ang receptor blockers with access to the brain reversed cerebrovascular inflammation by decreasing macrophage infiltration, normalization of endothelial NO synthase/inducible NO synthase ratio, reversal of heat shock protein upregulation, and reversal of increased TNF-α, IL-1β, and intercellular adhesion molecule.42 These observations beg the following question: are inflammatory conditions in the cardiovascular control regions of the brain a result of cytokines produced in the circulation and exerting their influence in the cerebral vasculature to initiate a cascade of events leading to increased neuronal activity and sympathetic tone? Alternatively, are cytokines produced within the brain to regulate neuronal activity? There is no conclusive answer to support or refute either contention at the present time. However, multiple studies support the latter view. They include the following: (1) increased expression of inflammatory cytokines has been demonstrated in cardiovascular relevant brain regions of various animal models of hypertension46–49 and in the heart failure model50,51; (2) intracerebroventricular infusion of inflammatory cytokines, such as IL-1α, activates the sympathetic nervous system and increases BP52; (3) overexpression of IL-10 in the central nervous system after intracerebroventricular administration decreases TNF-α, IL-1α, prostaglandin E2, and cyclooxygenase 2 in the PVN and ameliorates sympathoexcititation53; (4) adenovirus-mediated neuronal expression of IL-10 in the PVN attenuates chronic Ang II–induced hypertension54; (5) IL-6 in the NTS depresses the baroreceptor reflex gain55; (6) migratory inhibitory factor, an anti-inflammatory cytokine, has been shown to negatively regulate chronotropic actions of Ang II in the neurons.55 Thus, decreased migratory inhibitory factor expression in the PVN in response to Ang II may be associated with the development of high BP in the SHR48; and (7) numerous studies have demonstrated that inflammatory cytokines have profound effects on neuronal activity directly. For example, TNF-α and IL-1α increase neuronal firing frequency by binding to their respective receptors and the activation of nuclear factor κB.56 Consistent with this is the observation that intracerebroventricular infusion of a specific nuclear factor κB inhibitor (pyrroolidine dithiocarbamate) significantly attenuates Ang II–induced hypertension and inflammatory cytokines in the PVN.47 Cytokines can modulate neuronal activity via production of reactive oxygen species (ROS) and activation of inducible NO synthase. Resulting NO diffuses to adjacent tissues to modulate neuronal activity by influencing various ion channels.57,58 In addition, receptors for cytokines are found in several sites in the brain on multiple cell types, including neurons, microglia, and astrocytes.59 There is the possibility that a combination of both mechanisms (ie, contribution of peripheral cytokines via cerebral vasculature and generation of intrinsic cytokines within the brain) may contribute to the neuroinflammatory process in neurogenic hypertension. It appears that the inflammatory process in the cardiovascular regulatory regions of the brain is linked with modulation of the autonomic nervous system and the increased BP. However, we acknowledge that not all inflammation in the brain results in hypertension and propose unique inflammatory profiles relate to distinct disease states.60,61 Finally, animal and human studies indicate that hypoperfusion of the hypertensive brain can occur because of increased cerebral vascular resistance.60,61 The relationship between hypoperfusion and inflammation within brain microvasculature is undetermined, but one can trigger the other, thereby presenting a possible mechanism.

**Brain Microglia, Cytokines, and Neurogenic Hypertension**

Microglia, the resident macrophages of the brain, have attracted significant attention in recent years as a result of their role in various neuropathological conditions. They are activated rapidly in response to brain injury and produce a variety of inflammatory mediators, including cytokines. In ischemic stroke, for example, microglia exerts neurotoxic functions through production of ROS and cytokines. This damages the BBB integrity, leading to infiltration of inflammatory cells into the brain.62,63 Activated microglial cells are present in the central nervous system from patients with chronic neurodegenerative diseases, including Alzheimer disease, Parkinson disease, and amyotrophic lateral sclerosis.54,65 Their importance in these disease processes has been studied extensively.

The concept that microglial cells in the cardiovascular relevant brain regions may participate in hypertension-linked pathophysiology is derived, in part, from our studies, which show that chronic Ang II–induced hypertension is associated with increases in activated microglia in the PVN.49 This activation is associated with increases in the levels of PVN inflammatory cytokines. Similarly, we found an increase in
activated microglia in the PVN of the SHR (Figure 2). Further evidence in support of this concept is provided by our experiments with minocycline, an antibiotic that can cross the BBB and inhibit microglial activation.49 Intracerebroventricular infusion of minocycline attenuates Ang II–induced high BP, decreases PVN cytokines, and attenuates cardiac hypertrophy.49 Multiple examples in the literature indirectly support the role of brain microglia. Saavedra and colleagues66,67 have demonstrated that acute brain inflammatory response to bacterial endotoxin lipopolysaccharide results in the activation of microglia, not only in the cardiovascular-relevant brain regions (PVN and subfornical organ) but also in prefrontal cortex, hippocampus, and amygdala. This is associated with increases in brain expression of inflammatory cytokines and their receptors, adhesion molecules, and inducible NO synthase.66,67 Systemic administration of centrally acting AT1 receptor blockers decreases these responses. Carnevale et al68 studied interactions among hypertension, inflammation, and β-amyloid deposition in a hypertensive mouse with transverse aortic coarctation. They demonstrated that hypertension, per se, triggers microglia activation and neuroinflammation before the β-amyloid deposition takes place, suggesting that microglial activation precedes and may trigger neurodegenerative processes in Alzheimer disease. Finally, Lanz et al69 provided evidence that Ang II sustains brain inflammation in the experimental autoimmune encephalomyelitis mouse model of multiple sclerosis via the transforming growth factor β (TGF-β). Their studies indicate that neurons and microglial cells express AT1 receptors, which are primarily responsible for Ang II–induced increases in TGF-β production. Collectively, these observations provide persuasive arguments in support of the role in transmitting inflammatory signals that spread across the BBB into cardiovascular control regions of the brain.

**Visceral Control of Inflammation and Putative Alterations in Neurogenic Hypertension**

The brain is devoid of the “classic immunosurveillance system” but is in constant vigilance of the inflammatory status of the body by a reflex afferent feedback system. In recent years, the concept of “vagal immunoflex” has been introduced. This neuronal circuit involves activation of the vagal nerve afferents sensitive to systemic inflammation and reflex release of acetylcholine in organs such as the liver, spleen, bone marrow, and heart to dampen the tissue inflammation by reducing proinflammatory cytokines. The presence of specific acetylcholine receptors (nAchRα7) on inflammatory cells supports this anti-inflammatory role.70–72 In addition, vagal afferents can directly sense cytokine levels in the blood via specific cytokine receptors, such as IL-1R, present on the glomus cells adjacent to vagus nerve afferent endings within paranganglia located within the gastrointestinal tract, spleen, bone marrow, liver, and heart.73–77 Thus, the vagal immunoflex system presumably transmits signals to the NTS, the central site of vagal termination, before onward relay to dorsal vagal motoneurons.

It is concluded from the above discussion that the vagus nerve is vital in informing the brain of the peripheral immune status and controlling the activity of the immune cells. However, is this effective in hypertension? In hypertension, cardiac vagal tone and the cardiac vagal baroreflex gain are both depressed.78,79 Similarly, we propose that the anti-inflammatory function of the vagus is also suppressed in conditions of hypertension. The possibility that the raised levels of sympathetic activity (vasomotor nerves) associated with hypertension is also proinflammatory is a hypothesis at this point.

**Dysfunctional Autonomic Nervous System, Endothelial Repair, and Neurogenic Hypertension**

Could dysfunctional cardiovascular autonomic activity in neurogenic hypertension suppress endothelial repair? It is well accepted that endothelial dysfunction is an early event in hypertension-induced vascular pathophysiology. The healthy endothelial cells lining the blood vessels have both mechanical and functional roles, producing NO to promote vasodilation and reducing oxidative stress and inflammation. Because mature endothelial cells have a limited regenerative capacity, endothelial progenitor cells (EPCs) contribute to the repair and maintenance of endothelial damage to maintain normal vascular homeostasis in healthy individuals. In hypertension, however, dysfunctional endothelial cells produce ROS and other inflammatory molecules, which result in vasoconstriction, platelet activation, inflammation, and fibrosis.80–83 In addition, EPC numbers, migratory ability, and functions are all impaired in hypertension.84 A combination of these processes leads to accelerated vascular dysfunction and hypertension-associated pathophysiology; therefore, are EPCs from hypertensive dysfunctional and is there a neural control mechanism for EPC release and function that is altered in neurogenic hypertension?

Regarding the first question, EPC numbers and function have been inversely correlated in patients with cardiovascular disease, obesity, diabetes mellitus, chronic kidney disease,
and other immune diseases. Similarly, decreases in numbers, as well as their functions, have been demonstrated in human hypertensives and animal models of hypertension. Dysfunctional EPCs are associated with increases in EPC ROS, NADPH oxidase, and decreases in NO production. This view is supported by evidence that antihypertensive drugs, such as ACE inhibitors and Ang receptor type 1 blockers, increase EPC numbers and improve their function.90,91

Regarding the second question, release and function of EPCs from bone marrow appear to be neurally regulated. Bone marrow is densely innervated by the sympathetic nervous system, which stimulates the release of bone marrow-derived stem cells into the circulation. Finally, retrograde tracing studies with rabies virus have clearly indicated a connection between the bone marrow and many cardiovascular control regions of the brain.97

Considered together, these data suggest that dysfunctional sympathetic/parasympathetic regulation of bone marrow could lead to impaired EPC levels and their repair capacity in hypertension. An altered sympathetic (and/or parasympathetic) drive to the bone marrow may contribute to EPC dysfunction in hypertension. These proposals are supported by the following evidence: decrease in sympathetic activity to the bone marrow in diabetes mellitus is associated with decreases in circulating EPCs, which are also dysfunctional and, although it is generally considered that sympathetic activity is increased in hypertension, separate end organs can be differentially regulated by the sympathetic nervous system, and certain vascular beds reportedly receive decreased sympathetic activity in hypertension.98,99 It is tempting to suggest that a decreased sympathetic drive to the bone marrow could contribute to its dysfunction. In addition, a decreased parasympathetic (vagal) regulation of the bone marrow may instead contribute in its dysfunction (Figure 1).

Unifying Hypothesis

Synthesis of all of the available evidence has led us to propose the following hypothesis as a mechanism for neurogenic hypertension (Figure 1). Increase in peripheral Ang II leads to a cascade of events: AT1 receptors in the neurons of circumventricular organs are activated, resulting in increases in ROS,12,100,101 This leads to the activation of microglia in the PVN (Figure 2), which increases production of inflammatory cytokines, ROS, and other inflammatory modulators. These modulators would stimulate PVN neuronal activity, which, via brain stem (NTS and rostral ventrolateral medulla), would be reflected in modulation of autonomic nervous system, leading to increases in BP. An imbalance in sympathetic/parasympathetic activities also regulates bone marrow activity by increasing inflammatory cells and decreasing EPCs and their functions. Decreases in EPC numbers and their dysfunctions accelerate vascular dysfunction and damage. Increases in inflammatory cells would result in increased inflammatory mediators, which would exaggerate vascular dysfunction, leading to the development of pathophysiology of cardiovascular-relevant tissue characteristics of hypertension, as well as issues of hypoperfusion.61

The vascular arm of the neurovascular communication involves activation of AT1 receptors on the cerebral vessels, which have free access to increased levels of peripheral Ang II. Activation of these receptors stimulates cytokines, junctional adhesion molecule 1, ROS, and adhesion molecules. This interrupts the integrity of the BBB and enhances adherence of inflammatory cells causing leukocytes and cytokines to enter the brain parenchyma, contributing to microglial activation and inflammatory status of the PVN. Similar process could occur in other cardioregulatory brain regions.

Future Directions

Although some evidence, as presented in this review, exists in overall support of the above hypothesis, many important issues remain to be proven. Further evidence would be needed to provide conclusive validation for our hypothesis.

First, in our hypothesis, we have proposed a central role for the PVN. This is primarily based on our data that show activated microglia predominantly in this region (Figure 2). However, the simultaneous involvement of a similar mechanism in other brain regions (ie, NTS, rostral ventrolateral medulla, etc) cannot be ruled out and needs to be investigated.

Second, the hypothesis proposes that increases in activated microglia in the brain are both resident and derived from the peripheral inflammatory cells. There is no evidence to support this in hypertension. However, recent evidence from Chen et al has shown that defective microglia cause pathological grooming in Hoxb8 mutant mice. Bone marrow transplant from wild-type mice increases brain microglia and cures Hoxb8 mice of pathological grooming, indicating that activated microglia in this animal model results from immune cells in the peripheral circulation. In addition, Longo et al have demonstrated that a significant number of activated microglia observed in the hippocampus of status epileptus mice have bone marrow origin. Similar transplantation studies are needed to determine the origin of activated microglia in the brain in hypertension.

Third, are microglia targets of Ang II actions? It is without dispute that neurons in the cardiovascular regulatory brain regions, such as the PVN, express AT1 receptors, and Ang II interaction with these receptors profoundly affects neuronal activity by stimulating a cascade of signaling pathways. It remains to be proven, however, whether AT1 receptors are present on microglia, and, if so, how they contribute to increased neuronal activity in neurogenic hypertension. Recent studies have shown a colocalization of the AT1 receptor with Iba-1-positive cells, a marker for microglia in the brain of a mouse model of multiple sclerosis. In addition, TGF-β is colocalized with AT1 receptors in microglia. Furthermore, Lanz et al showed that experimental autoimmune encephalomyelitis-induced TGF-β expression is blocked by candesartan, an AT1 receptor blocker, concluding that microglia contains functional AT1 receptors. Evidence for this conclusion is provided by in vitro studies, which demonstrate that microglial cells in culture increase TGF-β expression in response to Ang II. How these receptors regulate generation
of inflammatory cytokines, ROS, and other mediators to influence neuronal activity remains to be investigated.

Lastly, we have hypothesized that a functional balance between the inflammatory cells and EPCs from the bone marrow is important in the maintenance of normal cardiovascular physiology. Its imbalance leads to vascular pathophysiology associated with hypertension. This concept needs to be further investigated.

**Perspectives**

Despite recent advances, it has been extremely difficult to successfully manage and control hypertension in a significant number of hypertensive patients. A majority of these unresponsive patients exhibit increased sympathetic drive and display neurogenic hypertension. Lack of underlying mechanisms involving neural-Peripheral communication associated with increased sympathetic activity has contributed to this. We have proposed a unifying hypothesis of a dysfunctional neural-immune-vascular communication pathway that may be responsible for neurogenic hypertension. Evidence to support/refute this hypothesis will be critical in the elucidation of the underlying impairment and exploring the mechanism for the advancement of neurogenic hypertension therapeutics.

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None.

**References**


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