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The Role of the Blood-Brain Barrier in Hypertension

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New findings:

- What is the topic of this review?

  This review highlights the importance of the blood-brain barrier in the context of diseases involving autonomic dysfunction such as hypertension and heart failure.

- What advances does it highlight?

  It highlights the potential role of pro-inflammatory cytokines, leukocytes and angiotensin II in disrupting the blood-brain barrier in cardiovascular diseases. Advances in our understanding of neurovascular unit cells, astrocytes and microglia, are highlighted with a specific emphasis on their pathogenic roles within the brain.

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Abstract

The blood-brain barrier (BBB) is a critical barrier that provides both metabolic and physical protection to an immune-privileged central nervous system. The BBB has been shown to be disrupted in hypertension. This review addresses the importance of the BBB in maintaining homeostasis in the context of diseases related to autonomic dysfunction such as hypertension. We highlight the potentially important roles of the immune system and neurovascular unit in the maintenance of the BBB, whereby dysregulation may lead to autonomic dysfunction in diseases such as heart failure and hypertension. Circulating leukocytes and factors such as angiotensin II and pro-inflammatory cytokines are thought to ultimately down-regulate endothelial tight junction proteins that are a critical component of the BBB. The specific mechanisms underlying BBB disruption and their role in contributing to autonomic dysfunction is not yet fully understood but is a growing area of interest. A greater understanding of these systems and advances in our knowledge of the molecular mechanisms causing BBB disruption, will allow for the development of future therapeutic interventions in the treatment of autonomic imbalance associated with diseases such as heart failure and hypertension.

Abbreviations. Ang II, angiotensin II; AT_1R, angiotensin receptor antagonist; BBB, blood-brain barrier; CCL2, chemokine ligand 2; IL, interleukin; MCP-1, monocyte chemoattractant protein-1; NTS, nucleus of the solitary tract; PVN, paraventricular nucleus of the hypothalamus; RAS, renin-angiotensin system; RVLM, rostral ventrolateral medulla; TNF-α, tumour necrosis factor-α.

Introduction

The blood-brain barrier (BBB) is critical in the regulation of central homeostasis as it is the primary barrier separating the peripheral circulation from the CNS (reviewed by Abbott *et al.*, 2010). Endothelial cells that express tight junction proteins form the main component of the BBB. These limit paracellular movement of substrates across the cerebral vasculature. These tight junctions are specialised protein complexes consisting of claudins, occludins and junctional adhesion molecules (Begley & Brightman, 2003). The on-going regulation of the BBB also involves astrocytes, pericytes and microglia, which collectively form part of the neurovascular unit (Obermeier *et al.*, 2013).

Astrocytic end-feet processes extend and contact endothelial cells to form the *glia limitans,* a membrane barrier that further limits movement across the BBB (Sofroniew & Vinters, 2010). This highly regulated system is essential to CNS homeostasis, allowing only minimal movement of small lipid-soluble molecules into the CNS. However, the BBB can become compromised in neurological diseases such as Alzheimer's (Kook *et al.*, 2013), Parkinson's (Chung *et al.*, 2013) and multiple sclerosis (Johnson *et al.*, 2014). These neurological diseases lead to both wide-spread and sparse BBB disruption. Interestingly, the BBB has also been shown to be disrupted in cardiovascular diseases such as neurogenic hypertension (Biancardi *et al.*, 2014).

Although the pathogenesis of hypertension involves dysfunction of the cardiovascular system, the autonomic nervous system and renin-angiotensin systems (RAS) are also major contributors to the disease. The pathogenesis of hypertension has been widely linked to the actions of the RAS, both within the periphery and in the brain. Infusion of angiotensin II (Ang II), a potent vasoactive peptide, elevates blood pressure and increases sympathetic output (reviewed by Young & Davisson, 2015). Moreover, Ang II induces oxidative stress and inflammation, which can further disease progression (Braga *et al.*, 2011). While the central actions of Ang II on autonomic function have long been realised (Scholkens et al., 1982), the downstream effects, including oxidative stress, neuroinflammation and BBB disruption, have only recently been uncovered.

In this report, we highlight the growing body of evidence that supports the hypothesis that BBB integrity is important for central autonomic control. We discuss the potential role of the immune system and its effects on BBB integrity and consider the role of the BBB and its constituent parts as potential therapeutic targets for the treatment of diseases that are, in part, mediated or exacerbated by autonomic dysfunction.

Blood-Brain Barrier Disruption in Hypertension

Angiotensin II and the Blood-Brain Barrier

Given the important role of Ang II in hypertension pathogenesis, our group previously showed that disruption of the BBB via hyperosmotic mannitol allows the entry of peripherally injected Ang II into the rostral ventrolateral medulla (RVLM) which activated tyrosine hydroxylase-expressing neurons involved in central cardiovascular regulation (Yao & May, 2013). Moreover, administration of losartan, an angiotensin receptor (AT_1R) antagonist, in part, prevented the activation of RVLM neurons. Recent studies have furthered these findings in a disease context using animal models of hypertension. These studies found that the BBB was disrupted in spontaneously and

renovascular hypertensive rats (Biancardi *et al.*, 2014). Importantly, they found that leakage of the 10 kDa fluorescent dextran was localised to central autonomic nuclei including the nucleus of the solitary tract (NTS), paraventricular nucleus of the hypothalamus (PVN) and the RVLM but not in the amygdala or somatosensory cortex. Furthermore, they showed that peripherally infused Ang II tagged with a fluorescent marker could enter these regions and gain access to neurons and microglia. This suggests that peripherally circulating factors can enter a leaky BBB and influence CNS signalling. Administration of losartan was found to prevent this disruption in the BBB and restore, in part, blood pressure homeostasis. However, administration of hydralazine, a direct vasodilator, failed to restore BBB integrity thus suggesting that BBB disruption, via an Ang II-AT_1R mechanism, may precede and contribute to the hypertension. Other studies have also shown that the BBB is disrupted in the hippocampus and corpus callosum of Dahl salt-sensitive hypertensive rats which resulted in cognitive deficits, while AT_1R blockade using olmesartan was also found to restore the BBB and improve cognitive function (Pelisch *et al.*, 2011). Ang II is thought to elicit these effects by acting on AT_1R on endothelial cells to increase BBB permeability. However, while BBB disruption is commonly associated with a decrease in tight junction protein expression, the levels of claudin-5 and occludin were not decreased by administration of Ang II *in vitro* (Fleegal-DeMotta *et al.*, 2009). This is contrast with *in vivo* studies on hypertensive rats that have reported decreased levels of occludin and zonula occludens-1 alongside disturbances in the ultrastructure of tight junctions, as determined by electron microscopy (Fan *et al.*, 2015). Given these findings, it is still unclear whether BBB disruption occurs prior to or is a result of hypertension. The mechanisms involved for allowing Ang II into brain are still unclear and therefore further studies are warranted to determine whether there is a causal relationship between BBB disruption and hypertension via an Ang II-mediated process.

Immune System and the Blood-Brain Barrier

A number of studies suggest that a chronic, low-grade inflammation is evident in hypertension (Zubcevic *et al.*, 2011; Coffman, 2011). In response to this low-grade inflammation, the adaptive immune system is activated causing an increase in circulating levels of inflammatory cells and mediators. Guzik and colleagues (2007) showed that mice which lacked both T and B cells have a blunted hypertensive response which supports a role of inflammation in hypertension. Circulating inflammatory molecules such as tumour necrosis factor-α (TNF-α), C-reactive protein, interleukin (IL)-6, monocyte chemoattractant protein-1 (MCP-1, also known as chemokine ligand 2 or CCL2) and IL-1β have all been found to be upregulated in both hypertensive patients and hypertensive animals models (Coffman, 2011). More recently, these pro-inflammatory markers have been found to

upregulated within the brain, suggesting a role of neuroinflammation in this disease (Winklewski *et al.*, 2015).

In the periphery, T cells and other lymphocytes release pro-inflammatory molecules which elicit cytotoxic effects on peripheral tissues leading to end-organ damage (Rudemiller & Crowley, 2017). However, whether these cells can enter the CNS is less clear. Although the CNS is immune-privileged, given that the BBB is compromised in hypertension (Biancardi et al., 2014), it is plausible that leukocytes or inflammatory mediators may extravasate into the CNS. Indeed, there is evidence that circulating leukocytes can infiltrate the CNS. In one study, Ang II-induced hypertension resulted in the accumulation of CD3 + T cells into the cerebral artery wall, suggesting that the vasculature was deleteriously affected (Meissner *et al.*, 2017). A more conclusive study showed that spontaneously hypertensive rats with reconstituted bone marrow from normotensive Wistar-Kyoto rats had decreased blood pressure and inflammation (Santisteban *et al.*, 2015). Furthermore, these bone marrow labelled cells were found within the PVN of naïve Sprague-Dawley rats when challenged with a continuous infusion of Ang II. Interestingly, these bone marrow derived cells were positive for the microglial marker, Iba1, suggesting the differentiation of these monocytes into functional macrophages/microglia, which could ultimately lead to the upregulation of a pro-inflammatory state within the brain. Although this study did not conclusively determine the mechanism by which these leukocytes entered the CNS, it is clear that these cells were able to enter from the circulation and into autonomic nuclei, such as the PVN.

Although these studies provide further evidence that the BBB is a critical interface, and its disruption may lead to altered brain function, autonomic imbalance and disease progression or exacerbation, the mechanisms remain unclear. A potential explanation for these findings can be attributed to the effects of circulating pro-inflammatory cytokines on the luminal side of the BBB. Pro-inflammatory cytokines are known to modulate BBB permeability. For example, administration of the chemokine, MCP-1, *in vitro,* was shown to decrease transendothelial electrical resistance, a surrogate marker for BBB disruption (Stamatovic et al., 2005). Further, Stamatovic and colleagues (2005) showed that MCP-1 caused a rearrangement of the actin cytoskeleton including reorganisation of tight junction proteins possibly via a Rho/Rho kinase pathway. However, MCP-1 is not the only pro-inflammatory mediator that can influence BBB permeability as both TNF-α and IL-6 contribute to BBB disruption via reactive oxidative species generation (Rochfort *et al.*, 2014). These studies demonstrated that the structural integrity of the tight junction complex can be affected by inflammatory mediators which ultimately increased the permeability. Given the upregulation of pro-inflammatory cytokines and leukocyte generation during autonomic dysfunction in hypertension

(Guzik *et al.*, 2007; Santisteban *et al.*, 2015) and heart failure (Mann, 2015), it is likely that these factors may mediate the initial dysfunction within the BBB, working alongside Ang II to disrupt the BBB and exacerbate the disease state, although further evidence is still required (Figure 1). These findings will be important to uncover novel pathways for circulating factors to enter the CNS; other than through the circumventricular organs which lack a functional BBB. Importantly, uncovering these pathways could reveal novel therapeutic targets to treat these autonomic-related cardiovascular diseases.

Neurovascular Unit and the Blood-Brain Barrier

There is clear emerging evidence that circulating inflammatory factors and inflammatory cells can enter the CNS. However, another major source of inflammatory molecules is thought to arise from within the brain itself; specifically from the glial components of the neurovascular unit: astrocytes and microglia (Heneka *et al.*, 2014; Sofroniew, 2015). Microglia are the brain's innate immune cells and their role in hypertension has increasingly been realised. For example, microglial inhibition with minocycline in hypertensive rats leads to a decrease in blood pressure (Shi *et al.*, 2010). Furthermore, ablation of microglia in transgenic mice has been found to reduce blood pressure and decrease levels of TNF-α and IL-1β in the PVN of Ang II-induced hypertensive mice (Shen *et al.*, 2015). Interestingly, adoptive transfer of activated microglia into naïve mice did not itself increase blood pressure; however, stimulation with Ang II resulted in a sustained pressor response when compared with sham controls. Collectively, these findings suggest a role of microglia in the maintenance of sustained hypertension; however, their role in the initiation of autonomic dysfunction still requires further investigation. Astrocytes have also been shown to work in concert with microglia as both cells release a myriad of pro-inflammatory mediators upon pathogen-induced activation. Once they have assumed a pro-inflammatory phenotype, astrocytes can release a host of chemokines and cytokines such as TNF-α, IL-1β, MCP-1 and IL-6 amongst many others (Sofroniew, 2015). Cumulatively, these factors are involved in the recruitment of leukocytes, monocytes and macrophages to the target site of injury. Interestingly, astrocytes also release vascular endothelial growth factors which have been shown to disrupt the BBB and increase leukocyte extravasation (Argaw *et al.*, 2012). In the spontaneously hypertensive rat, anatomical changes to the intermediate insular cortex, a cardiovascular related region was recently demonstrated (Marins *et al.*, 2017). Increased density of the NMDA receptor, angiogenesis, glial fibrillary acidic protein immunoreactivity and microglial activation were found to increase within this region, further supporting the theory that the neurovascular unit appears has a role in autonomic signalling in

hypertension. Furthermore, it was recently shown that astrocytes are involved in the signalling cascade of the sympathoexcitatory effects of Ang II in the PVN (Stern *et al.*, 2016). Here they showed that astrocytic glutamate transporters were predominantly responsible for maintaining glutamate homeostasis and thus had a critical influence on neuronal activity. Injections of Ang II were found to inhibit glutamate transporter activity on astrocytes within the PVN, therefore indirectly increasing neuronal activity and sympathetic outflow. In addition, our group has also recently found that pharmacological inhibition of astrocytes using sodium fluoroacetate causes an increase in blood pressure in normotensive animals which was coupled with a decrease in BBB permeability within the PVN (Setiadi et al., 2017), which further highlights the importance of this glial sub-type in autonomic regulation.

While the role of astrocytes and microglia and their effects on the BBB have been realised in neurodegenerative and neurological diseases, their role on the abluminal (brain) side of the BBB in hypertension is gaining significant interest. Given the diverse roles these glial cells play in maintaining homeostasis and the need for these cells and their effectors to operate in a fine-tuned balance of both pro- and anti-inflammatory responses, their effect on the BBB needs further detailed investigation. This growing area of investigation is exciting since the BBB is a prime target for therapeutic intervention. Given the BBB is an important interface between the brain and circulation, it plays a critical role in preserving central homeostasis and as such is an important target for novel clinical targets. Further investigation of the downstream molecular mechanisms will be critical in uncovering the importance of BBB integrity and allow for targeted interventions, which could ultimately successfully treat or limit the progression of autonomic diseases such as hypertension and heart failure.

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Author Contributions

All authors, AS, WSK, KE and STY conceived and discussed the content of the manuscript. AS drafted the manuscript and figure while WSK, KE and STY critically revised the intellectual content. All authors approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

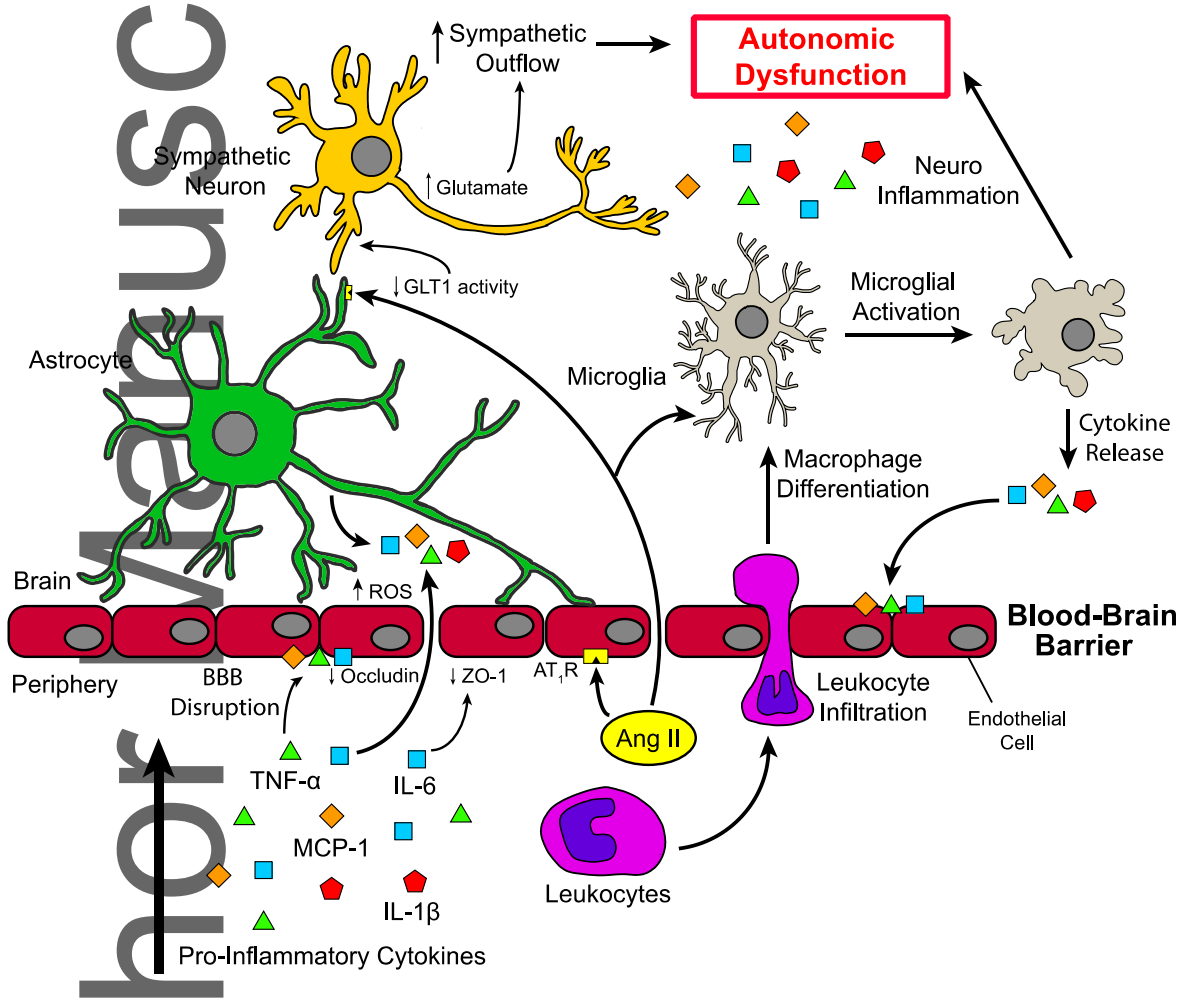

Figure 1. Proposed mechanism whereby the blood-brain barrier (BBB) is disrupted leading to autonomic dysfunction. Circulating pro-inflammatory cytokines and Ang II from the periphery acts on AT_1R on the BBB endothelium, which causes disruption in TJ proteins, occludin and ZO-1. Circulating leukocytes gain access to the brain and differentiate into functional microglia. These release pro-inflammatory cytokines and cause neuroinflammation. Ang II can act directly on astrocytes to inhibit GLT1 activity to increase glutamate that activates sympathetic neurons and

autonomic dysfunction. The BBB is further disrupted due to release of pro-inflammatory cytokines from astrocytes and microglia. Ang II, angiotensin II; AT_1R, angiotensin receptor antagonist; GLT1, glutamate transporter 1; IL, interleukin; MCP-1, monocyte chemoattractant protein-1; ROS, reactive oxidative species; TNF-α, tumour necrosis factor-α; TJ, tight junction; ZO-1, zonula occludens-1.

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