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## **HIF-1 at the blood-brain barrier: A mediator of permeability?**

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## **Abstract**

The importance of the blood-brain barrier (BBB) in maintaining brain homeostasis cannot be better appreciated than during disease states, where disruption of its function is associated with dramatic detrimental clinical outcome. For decades, neuroscientists and neurobiologists investigated most neurological diseases under the prism of a neuro-centric view, considering the contribution of non-neural components of the CNS (BBB, choroid plexus) negligible or even irrelevant. However, recent reviews have highlighted the importance of BBB breakdown in major neurological diseases.

Hypoxia, as well as hypoxia/reoxygenation, is a key component of many neurological diseases and has been shown to significantly contribute to barrier disturbance and dysfunction. Since the master regulator of the hypoxic response, hypoxia inducible factor 1 (HIF-1), is a key determinant for adaptation of cells and tissues to oxygen deprivation, it is likely that this transcription factor also plays a key role in barrier permeability. The possible future use of HIF-1 stabilizers for treatment of diseases characterized by oxygen deprivation to increase neuronal/cell survival means this question is now very pertinent.

This review will focus its attention on the role of HIF-1 in BBB breakdown following hypoxic/ischemic injury and the implications for such therapies in a clinical setting.

## 1. The blood-brain barrier (BBB)

### 1.1. Introduction to the blood-brain barrier (BBB) An overview

The central nervous system (CNS) constitutes one of the most important systems present in vertebrates, tightly regulating both vegetative and cognitive functions. In vertebrates most of the CNS is formed by neurons and glial cells (e.g. astroglia, oligodendrocytes and microglia). Due to their excitatory nature and their inability to further divide, neurons require a chemically defined and stable extracellular environment, sheltered from any sudden changes in composition. The ability to maintain such a microenvironment is achieved exclusively by the presence of the blood-brain barrier (BBB). The first documented description of the BBB was attributed to Paul Ehrlich [1] at the end of the 19<sup>th</sup> Century, as he described the absence of chemical dye penetration within the CNS. However, the cellular and molecular nature of the BBB remained unclear for more than 80 years until the publication of two seminal studies by Reese and colleagues. In the first study [2], the authors described the presence of the barrier at cellular junction between brain endothelial cells (ECs) lining the cerebral vasculature. Later on, the same authors demonstrated the presence of “tight junction” (TJ) complexes between EC cell junctions that were responsible for the barrier phenotype [3].

Although the BBB denomination was originally limited to brain ECs, the current consensus rather defines it as a multicellular neurovascular unit [4, 5]. The BBB is formed by a monolayer of specialized brain ECs harboring TJs complexes at their cellular junctions. These brain ECs are in direct contact with brain pericytes [6, 7] and separated from the brain parenchyma by two layers of extracellular matrices (ECM). The inner layer is formed by the vascular basement membrane localized at the EC basolateral side and is shared with neighboring pericytes. The outer layer is formed by the *glia limitans* that surrounds or ensheaths the cerebral vascular tree. Interestingly, in larger vessels these two matrix layers may also be separated by a perivascular space and populated by perivascular cells, particular cell types capable of macrophage activity [8, 9]. The main function of this space remains unclear, however it appears to play an important role in neuroinflammatory diseases [10]. The *glia limitans* is formed by polarized astrocyte end-feet processes that directly contact and cover the vast majority of the microvasculature

[11]. Finally, neurons and microglia contact the brain microvessels although the specific nature of these additional interactions is also debated.

### *1.2. The BBB: the gatekeeper of CNS homeostasis*

The BBB plays a crucial role in maintaining CNS homeostasis by acting as a formidable “gatekeeper”, regulating the entrance of any chemical and biological entities into the CNS and vice-versa. Firstly, the presence of very “tight” TJ complexes eliminates the passive diffusion of solutes across the endothelium. Secondly, non-selective transport of small solutes by pinocytosis is virtually inexistent as brain ECs have a very low pinocytic activity. Therefore, the entrance of nutrients (glucose, amino acids etc) through the BBB can only be achieved by the recruitment of specialized solute carriers [12]. In contrast, lipophilic compounds (e.g. drugs, poisons) may reach the brain parenchyma easily via passive diffusion although the majority of these compounds have only limited bioavailability since the BBB presents an array of various ATP-binding cassettes (ABC) efflux transporters such as P-glycoprotein (P-gp, ABCB1), breast cancer resistance protein (BCRP, ABCG2) and multidrug-resistant polypeptides (MRPs) at their cell surface [13-16]. These transporters are highly efficient in removal/efflux of any foreign substances that have managed to penetrate the vascular system from the circulating blood such as drugs and pharmaceuticals. Substrates of these transporters have a broad diversity in their chemical structures, making it very difficult to predict whether a newly designed drug candidate may avoid the efflux pump - indeed current success rates are exceedingly low. In addition, brain ECs are also capable of drug metabolic activity by the presence of certain cytochrome P450 enzymes such including CYP1B1 and CYP2U1 [16] which may therefore further compromise the diffusion of any spared molecules. The ability of the BBB to efficiently metabolize, efflux or exclude the entrance of foreign substances to the brain means that it also represents a formidable obstacle for drug entry and treatment of brain pathologies. Taken together, the essential “gatekeeper” function of the BBB is a blessing and a curse for the CNS: it prevents entrance of harmful agents capable of severely compromising brain integrity but also prevents the entrance of pharmaceutical

drugs that could restore neuronal function and promote repair mechanisms following injury.

Accumulating experimental evidence supports the hypothesis that opening of the BBB triggers a chain of events leading to neuronal dysfunction and damage resulting in neurological disease [4, 5, 17-19]. When coupled with previous brain insults, additional BBB disruption could have serious detrimental consequences for patient outcome.

### *1.3. Tight junction complexes: the CNS great wall*

Endothelial cells that line the cerebral capillaries form the anatomic basis of the BBB in higher organisms. Unlike the endothelium of other vascular beds, specialized cerebral microvessel endothelial cells have very low permeability due to the presence of highly organized junctional complexes called tight junctions (TJs). TJ complexes represent highly intimate cell contacts and ensure stringent regulation of CNS homeostasis by severe restriction of the paracellular diffusional pathway between the endothelial cells and substances and/or cells within the circulating blood. These complexes are located within specific TJ strands bordering the basolateral side and brain ECs present three major types of TJ proteins: occludin [20, 21], claudins [22-24] and junctional adhesion molecules (JAMs) [25]. Occludin, claudin and JAM interactions occur through homotypic interactions [26, 27] however recent suggestion of heterotypic occludin interactions additionally imply a certain plasticity and dynamism at the BBB [27].

Occludin is a 65kDa tetraspan membrane protein [20] encoded by the *OCLN* gene. Its expression is mostly restricted to epithelial and endothelial cells. Very interestingly, occludin-deficient animals did not present major barrier leakage even at the BBB [28]. However abnormal calcification around cerebral vasculature was noted in these mice suggesting that occludin may regulate diffusion of bivalent cations such as calcium or magnesium. It is currently suggested that occludin plays a more permeability-regulating role by incorporating itself into the claudin-based strands (reviewed by [29]). The mechanism by which this occurs, and indeed the precise role(s) of occludin remain to be elucidated. Soon after cloning occludin the same authors described an additional class of TJ proteins called claudins [30]. Claudins are 20-27kDa tetradomain membrane proteins

encoded by 23 different *CLDN* genes in human. Until now, four major claudins have been described at the BBB: claudin-1 [31], claudin-3 [32], claudin-5 [23] and claudin-12 [33]. Evidence suggests that the claudins constitute the backbone of TJ strands at the BBB [29]. Increased expression of claudin 5 in rat brain capillary endothelial cells *in vitro* resulted in decreased monolayer permeability [34]. Unlike occludin<sup>-/-</sup> animals that showed no major vascular leakage, claudin-5<sup>-/-</sup> mice rapidly died after birth. Although no macroscopic vascular leakage was observed in these animals, Nitta and colleagues [33] noted an increased permeability to molecules with a molecular weight below 800 Da, suggesting that claudin-5 may infer the highest tightness to the “tight junctions”. The effect of deletion of other claudins on the BBB remains undocumented as these animals rapidly die *in utero* or during the early phase post-partum due to major epithelial lesions. Finally, the third class of protein described at the TJ complexes are represented by the JAMs [25]. Unlike occludin and claudins, JAMs belong to the immunoglobulin (IgG) superfamily and present two-extracellular IgG-like domains. Endothelial cells express all three different isoforms of JAMs: JAM-A, JAM-B and JAM-C [35]. JAMs play important roles in modulating barrier function in non-BBB endothelial cells as well as leukocyte-endothelial interactions. Although the importance of JAMs on BBB function remains largely unclear decreased JAM-A protein levels following BBB breakdown [36] and an increase in soluble JAM-A following BBB injury [37] suggests that JAM-A shedding may constitute a biomarker of BBB injury.

Similar to other cell junction proteins, TJ membrane proteins interact with the actin cytoskeleton by soliciting the recruitment of zonula occludens proteins, classically referred as ZOs [38]. ZO proteins belong to the membrane associated guanylate kinase (MAGUKs), as they contain one or several PDZ, src-homology3 (SH3) and guanylate kinase (GK) domains. Both ZO-1 and ZO-2 expression at the BBB were demonstrated in the literature [39, 40], whereas proven ZO-3 expression remains undocumented. Interactions between ZO proteins and TJ membrane proteins occurs through their PDZ domains, whereas interactions with the cytoskeleton occurs through their C-terminus via their actin-binding regions (ABRs). In addition to these distinct domains, ZO proteins present several nuclear localization signals [38] suggesting a certain ability to shuttle between the cytoplasm and nucleus. Thus ZO proteins may act as transcription factors in

addition to their structural scaffold function although the nature of ZO target genes and their relevance at the BBB remains unknown.

## **2. Hypoxia and BBB function**

### *2.1. Hypoxia induces BBB disruption*

Reduction of oxygen levels such that supply fails to meet demand is termed hypoxia. Hypoxia is a strong stimulus for various physiological processes particularly during development but is also a major cause or consequence of injury and contributes to progression of many different diseases and pathologies. To ensure hypoxic survival cells must be able to adapt to oxygen deprivation and switch from aerobic to anaerobic metabolism until oxygen levels are restored to manageable levels. Notably resting oxygen levels, and sensitivity to oxygen deprivation, differ widely in various tissues and organs meaning that hypoxic exposure can have differential effects based on the tissue or cells being studied. The CNS constitutes a system that utilizes an unparalleled degree of physiological resources. With an average blood vessel surface of  $10\text{m}^2$  [41] it solicits 15-20% of total cardiac output and 20% of the arterial  $\text{O}_2$  input on its own under resting conditions [42]. In addition, it heavily relies on glucose as a source of energy by consuming ~20% of daily glucose intake [18]. Indeed, such extreme consumption underlies a heavy dependence of the cerebral tissue on constant  $\text{O}_2$  and glucose perfusion. Thus a rapid change in environmental or local  $\text{O}_2$  levels may result in dramatic consequences for CNS homeostasis and BBB integrity. Indeed hypoxia/ischemia may constitute the most frequent cerebrovascular event leading to BBB breakdown.

The effects of hypoxia at the BBB have been extensively investigated. Hypoxia, as well as hypoxia/reperfusion stress and cerebral ischemia have been shown to alter localization and expression of the key junctional proteins ZO-1 and occludin at the BBB in both *in vivo* and *in vitro* BBB models, and correlates with increased paracellular permeability and edema [40, 43-46]. The effect of hypoxia on BBB function was also demonstrated to reduce claudin 5 expression levels and increase paracellular permeability of low molecular weight compounds after exposure of brain endothelial cells and retinal flatmounts to hypoxia [47]. The disruption of the BBB in hypoxic conditions is multi-



factorial and may involve factors such as enhanced production of vascular endothelial growth factor (VEGF), nitric oxide (NO) and inflammatory cytokines (reviewed by [48, 49]. Increased cytokines and subsequent up-regulation of endothelial and neutrophil adhesion molecules lead to leucocyte adhesion and transmigration across the endothelium and the BBB creating a positive feedback loop that further enhances vascular damage [49].

Notably, ischemia or hypoxia-induced alterations in BBB TJs have not been observed in all studies. This may be related to differences in the severity of hypoxia within different areas of the brain following insult, the differential cell-response of surrounding cell types as well as the duration of the injury [50]. Understanding the inter-related and complex contributions of these parameters to the modulation of barrier function may be of significant relevance in the design of future therapeutics.

### *2.2 Hypoxia-mediated HIF-1 signaling*

Hypoxia induces a variety of signaling pathways. The most widely studied mediators of the hypoxic response are the family of transcription factors known as hypoxia inducible factors (HIFs). There are 3 known members of the HIF family, namely HIF-1, 2 and 3 with HIF-1 being the most well characterized and generally considered the master regulator of the hypoxic response. HIF-1, mediates many adaptive endogenous mechanisms during hypoxic brain injury by transcriptional activation of specific target genes that function to restore oxygen supply.

HIFs are heterodimeric transcription factors consisting of an oxygen-inducible  $\alpha$  subunit (HIF $\alpha$ ) and an oxygen-independent subunit (HIF $\beta$  also known as ARNT) [51-53]. These subunits are differentially localised with HIF $\alpha$  being expressed in the cytoplasm whereas ARNT is a nuclear protein. Under normal conditions, i.e. in the presence of oxygen, the HIF $\alpha$  protein is constantly degraded due to hydroxylation of specific proline residues by enzymes called prolyl hydroxylases (PHDs) [54-57]. This modification leads to recognition by the von hippel lindau protein, ubiquitination and degradation by the E3 ligase machinery. When oxygen is reduced and becomes limiting the PHD enzymes are inhibited. As a result HIF $\alpha$  is no longer degraded but accumulates and after

phosphorylation is transported to the nucleus where it binds ARNT forming the functional HIF protein. Thereafter the heterodimer forms a complex with a number of other proteins and subsequently binds the hypoxic response element (HRE) in the promoter of target genes inducing their expression. To date a large number of HIF target genes have been identified, many being involved in the switch from aerobic metabolism to glycolysis as well as angiogenesis and erythrocytosis i.e. changes that reduce energy consumption and promote re-establishment of oxygen delivery thus facilitating cellular adaptation to oxygen deprivation, as well as a number of target genes involved in tissue repair [55, 58, 59].

Thus HIF-1 is largely considered to be essential for cellular survival during injury and has been reported to protect neurons from apoptosis caused by oxidative stress and focal cerebral ischemia [60-62]. Stimulation of HIF-1 $\alpha$  upon hypoxic preconditioning or chemical induction of HIF-1 $\alpha$  was also shown to induce HIF-1 target pro-survival genes such as VEGF and Epo resulting in increased cell survival [60, 63, 64] and neuroprotective effects (reviewed by [65]). HIF-1-induced angiogenesis and glycolytic metabolism also increased delivery of oxygen and nutrients that are critical for cell survival under hypoxic/ischemic conditions [63]. Furthermore, neuron-specific knockdown of HIF-1 $\alpha$  was demonstrated to increase tissue damage and reduce survival of mice subjected to middle cerebral artery occlusion [66].

Although HIFs are essential for cellular adaptation to reduced oxygenation, over the last few years it has become apparent that these transcription factors can act as double-edged swords. Indeed a wealth of contrasting data suggests that in addition to cellular adaptation and survival, HIFs also contribute to activation of cellular processes that lead to apoptosis and necrosis. Several groups have reported detrimental effects of HIF-1 in cerebral ischemia. For instance, Halterman et al. [67] reported that HIF-1 $\alpha$  coordinated the activity of p53 in driving ischemia-induced delayed neuronal death instead of providing neuroprotection. HIF-1 was shown *in vitro* to mediate hypoxia-induced growth arrest and apoptosis [68] and regulate the expression of proapoptotic family members such as BNIP3 and caspase-3 that are increased following cerebral ischemia [69-72]. Furthermore brain-specific knockdown of HIF-1 $\alpha$  reduced ischemic damage in knockout mice and was neuroprotective [73].

Overall current data suggests that mild or acute hypoxia induces adaptive gene expression such as EPO, Glut1 and VEGF, whereas severe or sustained hypoxia HIF-1 $\alpha$  can lead to activation of prodeath genes, such as BNIP3, COX2, or p53 stabilization (reviewed by [74]). Thus, HIF-1 can activate transcription factors and signaling pathways with both pro-death and pro-survival functions. The outcome of HIF-1 induction appears to be dependent on the duration [67], the pathological stimuli and the cell type in which it is induced [75]. Thus it is apparent that during oxygen deprivation cell-specific temporal-spatial modulation of crucial HIF-1 signaling pathways is a key determinant of functional outcome. It is also essential to keep in mind that activation of the pathway under certain circumstances may also have negative outcomes – especially if the system is chronically activated/induced.

Besides regulation by hypoxia, other signaling pathways can also modulate HIF activation. An example is evidence for a role of mitochondrial ROS in cellular oxygen sensing in at least some cell types [76]. The contribution of oxygen sensing, redox status and a variety of molecular factors and pharmacological agents to HIF stabilization has recently been reviewed by [74, 77].

### **3. HIF-1 signaling at the BBB**

#### *3.1 Effect of HIF-1 on barrier permeability*

As stated above it is known that hypoxia compromises barrier integrity however the precise mechanisms that mediate barrier dysfunction remain largely unknown. The role of HIFs and their target genes in major cellular alterations and adaptations in response to oxygen deprivation suggests they may be instrumental modulators of BBB integrity. Indeed current data from our group and others suggest that HIF-1 is a likely mediator of barrier disruption. A possible role for either HIF-2 or 3 at the BBB is yet to be addressed. A study by Witt et al first suggested that transcription factors such as HIF-1 and NF $\kappa$ B are upstream mediators of TJ protein alterations during hypoxia and H/R, which may involve VEGF induction and expression. Indeed VEGF is a strong inducer of vascular permeability and increased VEGF levels positively correlate with changes in TJ redistribution of zona occluden-1 (ZO-1) and occludin, as well as with alterations in the

actin cytoskeleton both *in vivo* and *in vitro* [78-81]. Yeh et al [82] subsequently demonstrated that 3-(5'-hydroxymethyl-2'-furyl)-1-benzylindazole (YC-1) an inhibitor of HIF-1 $\alpha$  was able to prevent increased permeability in adult rat brain endothelial cells in response to chemical hypoxia likely through inhibition of HIF-1 $\alpha$  accumulation and VEGF production. Pretreatment with YC-1 also seemed to reduce ischemia/reperfusion-induced increase of BBB permeability and HIF-1 $\alpha$  accumulation in a rat *in vivo* model [82]. Elevation of HIF-1 $\alpha$  was shown to be harmful in cerebral ischemia after 2 h of MCA occlusion, and inhibition of HIF-1 $\alpha$  and VEGF by 2ME2 and D609 could protect against brain damage by reducing neuronal expression of BNIP3, and cleaved caspase 3 during stroke [74]. Acute inhibition of HIF-1 $\alpha$  (when administered within a 3 hour window) was also neuroprotective in neonatal hypoxic-ischemic injury by preserving BBB integrity and reducing brain edema [83]. Interestingly however most of the *in vivo* studies have not demonstrated convincingly that the crucial HIF modulation takes place in the endothelial cells, indeed in most cases the neurons were the most sensitive and robustly upregulated HIF-1.

Very recently it was demonstrated that despite ameliorating ischemia-induced BBB disruption (determined by Evans blue leakage) YC-1 did not alter brain edema formation and significantly exaggerated ischemic brain damages in terms of infarct volume and mortality as evaluated by MRI and histological staining [84]. The data indicates that BBB protection resulting from HIF-1 inhibition by YC-1 contributes little to the overall brain tissue injury induced by cerebral ischemia – a finding that needs to be further investigated. However the study clearly implies that the presence of HIF-1 is critical in promoting neuronal survival during ischemia/reperfusion and HIF-1 modulation may have differential effects on ischemic outcome and BBB permeability.

Notably, detrimental effects of HIF-1 on BBB function may not only be limited to stroke-related events. In an *in vivo* model of traumatic brain injury (TBI) brain edema was significantly decreased after inhibition of HIF-1 $\alpha$  [85]. HIF-1 $\alpha$  activation in the brain of dystrophic mouse (model for Duchenne muscular dystrophy) was also suggested to be partly responsible for both BBB opening and increased angiogenesis through reduced protein levels and increased phosphorylation of ZO-1 as well as an up-regulation of VEGF and VEGFR-2 expression [86]. HIF-1 $\alpha$  was shown to be involved in brain edema

formation and BBB disruption via a molecular signaling pathway involving AQP-4 and MMP-9 in a subarachnoid hemorrhage model [87]. Other data strongly indicates that HIF-1 plays an important role in high glucose-induced BBB dysfunction. Upregulating HIF-1 activity by cobalt chloride increased the paracellular permeability of endothelial cells exposed to normal glucose whereas downregulating HIF-1 activity, by HIF-1 $\alpha$  inhibitors and HIF-1 $\alpha$  specific siRNA, ameliorated the redistribution of occludin and ZO-1 and increased permeability induced by high glucose [88]. The involvement of HIF-1 in impaired junction assembly in the kidney has also been reported [76]. The precise mechanisms by which HIF-1 modulates TJ proteins are still to be fully elucidated. Hypoxia also rapidly stimulates cytoskeletal reorganisation *in vitro* [89, 90] and *in vivo* [48, 91] and attachment of TJ proteins to the actin cytoskeleton via accessory proteins [92] means that barrier permeability is also closely linked to such changes. Cytoskeletal rearrangements also result in cellular movement, such as endothelial migration leading to angiogenesis and retraction of astrocyte endfeet from vascular walls, both of which contribute to barrier breakdown. Whether these effects are HIF-1 dependent or independent is still to be addressed.

### *3.2 Cell-specificity of HIF-1 mediated responses and their effect on BBB integrity*

Although current concepts advocate that conserved hypoxic adaptive mechanisms occur in many different cell types of the brain, specific temporal-spatial modulation of crucial pathways such as HIF signaling may be the key determinant of functional outcome. Thus the impact of activation of the HIF-1 pathway on the response of individual BBB cells and subsequent barrier function is a pertinent issue that needs to be tackled.

Our studies clearly indicate that individual responses of barrier cells to O<sub>2</sub> deprivation define the outcome of hypoxic injury and the integrity of the BBB [50, 93]. The contribution of HIF-1 induction by barrier modulating cells, astrocytes and pericytes as well as neurons themselves, to barrier stability is still a largely open question. Unlike many other cells, astrocytes are very resistant to hypoxic stress. We showed that severe oxygen deprivation is required to induce HIF signaling and modulate subsequent survival and proliferation in astrocytes [94]. However our data also indicates that VEGF induction

during hypoxia in these cells likely occurs through HIF-1 dependent and independent mechanisms. Our current investigations advocate that pericytes are also hypoxia-resistant cells (unpublished data *Engelhardt & Ogunshola et. al.*) although fully comparable studies are still to be completed. Notably, astrocytes and pericytes have differential responses to hypoxia that are important for barrier regulation depending on the duration and severity of insult [50]. Although impairment and/or alteration of either astrocyte or pericyte function results in microvascular damage and accelerates neuronal death [49, 95, 96], the contribution of activation of HIF-1 signaling in these cells to BBB leakage and central nervous system edema formation remains largely unknown. Interestingly it was recently demonstrated that although loss of HIF-1 $\alpha$  function in neurons reduced neuronal viability during hypoxia, selective loss of HIF-1 function in astrocytes markedly protected neurons from hypoxic-induced neuronal death [75]. Notably, a systematic *in vivo* and *in vitro* investigation of cell-specific responses mediated via HIF-1 is warranted to provide detailed knowledge of physiological and pathological barrier modulation, and define future targets for development of rational therapeutic approaches that optimize recovery.

#### **4. HIF-1 stabilization as a therapeutic target**

Therapeutic activation of HIF-1 is likely to mimic, at least in part, the effects of hypoxia preconditioning. Indeed it has been suggested that certain protective agents in stroke may act via HIF induction (reviewed by [76]) but as is clear from the studies outlined above a major caveat is that not all consequences of HIF activation may be beneficial and some could even be deleterious. In general the therapeutic potential of development and use of small molecule HIF stabilizers (PHD inhibitors) to improve cell survival after injury is gaining popularity in many different fields. Such drugs could provide significant protection for a variety of different cells during injury and pathological situations such as stroke, TBI etc. Since the first indications are that HIF-1 may disturb barrier function the applications of such therapeutics must be treated with caution. Enhanced edema, and influx of other blood-borne molecules as a result of increased BBB permeability, will increase intra-cranial pressure and the transport of potentially detrimental substances into

the brain parenchyma. Thus the adverse effects of reduced barrier integrity must be carefully assessed when administering HIF-1 stabilizing drugs. It must also be emphasized however that, as suggested by *in vitro* and *in vivo* studies, the duration of the stabilization of HIF-1 and the regions being targeted will likely be instrumental in obtaining a positive outcome during and after treatment and must also be taken into consideration. A number of reviews on the effects and use of PHD inhibitors and HIF-1 stabilizers as therapeutics have recently been published [76, 97, 98].

On the other hand, it is feasible that such drugs could be used to induce a partial opening of the barrier and thus facilitate drug entry into the brain parenchyma. Such a strategy using combination therapies could have high benefit under particular scenarios. Indeed selective opening of the endothelium tight junctions to facilitate drug delivery to the brain is also an area of intense research since delivery of therapeutics to the CNS remains highly challenging [99, 100]. Of course the feasibility of such an approach, although very attractive, remains to be properly researched to ensure exploitation of the benefits of BBB modulation, while minimizing the potential damage.

## **Conclusion**

Maintenance of BBB integrity and thus brain homeostasis is crucial for brain cells, and highly sensitive neurons in particular, to be able to function. During hypoxia/ischemia the BBB is compromised resulting in alterations that can have significant detrimental effects. Thus identification of the mediators of barrier dysfunction may provide not only important avenues to prevent barrier permeability but also perhaps ways to selectively modulate barrier function and facilitate the passage of protective drugs and therapeutics. Current data suggests that HIF-1 may represent such a mediator but more knowledge of the regulation of specific barrier properties during different injury paradigms and windows of opportunity is required. Better assessment of the positive versus the negative effects of acute versus chronic stabilization of HIF-1 is also needed. Overall considering its multifunctional role, differential effect on different cells and double-sword mode of action it seems unlikely that HIF-1 stabilization on its own will be the magic solution to

improving brain cell survival after injury - but perhaps combination therapies will provide significant gains. As always many questions are still to be answered.

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