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REVIEW ARTICLE

Transient Opening of the Blood-Brain Barrier by Vasoactive Peptides to Increase CNS Drug Delivery: Reality Versus Wishful Thinking?

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Abstract: ***Background*:** The blood-brain barrier inhibits the central nervous system penetration of 98% of small molecule drugs and virtually all biologic agents, which has limited progress in treating neurologic disease. Vasoactive peptides have been shown in animal studies to transiently disrupt the blood-brain barrier and regadenoson is currently being studied in humans to determine if it can improve drug delivery to the brain. However, many other vasoactive peptides could potentially be used for this purpose.

***Methods*:** We performed a review of the literature evaluating the physiologic effects of vasoactive peptides on the vasculature of the brain and systemic organs. To assess the likelihood that a vasoactive peptide might transiently disrupt the blood-brain barrier, we devised a four-tier classification system to organize the available evidence.

***Results*:** We identified 32 vasoactive peptides with potential blood-brain barrier permeability-altering properties. To date, none of these are shown to open the blood-brain barrier in humans. Twelve vasoactive peptides increased blood-brain barrier permeability in rodents. The remaining 20 had favorable physiologic effects on blood vessels but lacked specific information on permeability changes to the blood-brain barrier.

***Conclusion*:** Vasoactive peptides remain an understudied class of drugs with the potential to increase drug delivery and improve treatment in patients with brain tumors and other neurologic diseases. Dozens of vasoactive peptides have yet to be formally evaluated for this important clinical effect. This narrative review summarizes the available data on vasoactive peptides, highlighting agents that deserve further *in vitro* and *in vivo* investigations.

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1. INTRODUCTION

The failure to improve therapy for central nervous system (CNS) diseases is largely due to inadequate drug penetration of the blood-brain barrier (BBB) [1-5]. The BBB is an evolutionarily conserved structure where astrocytes, microglia, and neurons collectively form the neurovascular unit (NVU) to protect the CNS through strict regulation of the movement of molecules, ions, and cells between the blood and the tissues of the CNS [6-9]. A variety of invasive and medicinal approaches have been studied in an effort to disrupt the BBB and enhance CNS drug delivery [9-12]. Unfortunately, these approaches have had minimal impact on human diseases [12]. Vasoactive peptides (VAPs) induce physiological activity in blood vessels. Some of these have vasodilating or vasoconstricting properties, which can have different effects depending upon the vascular bed, and others are mediators of vascular permeability [13, 14]. Three of these VAPs (regadenoson, adenosine, and labradimil) transiently open the BBB in animals allowing more CNS drug delivery, and one (regadenoson) is currently being investigated for this indication in humans (NCT03971734) [12, 15, 16]. The vast majority of VAPs have not been formally evaluated preclinically to determine their effect on the BBB. Studying these agents for this purpose presents a unique opportunity. A positive signal would potentially improve the treatment of a broad range of oncologic, infectious, psychiatric, degenerative, and other neurologic diseases.

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2. BACKGROUND

2.1. The Blood-Brain Barrier

The BBB was initially described in 1885 when Paul Ehrlich injected rats with alizarin blue and found that this blue tracer was present in virtually all systemic tissues except for the brain [6]. This evolutionarily conserved structure is present throughout the animal kingdom, including insects [6]. In humans, the brain vasculature has 12 m^2 of the endothelium, with 80% of the brain capillary surface covered with pericytes [17]. The BBB is comprised of vascular smooth muscle cells and endothelial cells, which are supported by surrounding pericytes, astrocytes, microglia, and neurons that make up the NVU. Together, they tightly regulate access of molecules, ions, and cells between the blood and the CNS [6–9, 18]. Compared to the periphery, endothelium within the CNS is particularly enriched for cell-cell junctions, including adherens junctions and tight junctions, which provide structural stability and regulate paracellular transport [19, 20]. CNS endothelium limits immune cell entry through a relative paucity of leukocyte adhesion molecules and has low levels of transcytosis [6, 21]. The BBB regulates immune cell trafficking into the brain, affecting the efficacy of immunotherapy in primary and metastatic brain cancers [22, 23]. Pericytes are necessary for the development of the BBB, and loss leads to the breakdown of the BBB and reduces cerebral blood flow [24]. The microvessels and capillaries of the brain are wrapped by pericytes with a ratio of 1:3 to endothelial cells [19]. Pericytes are infiltrated with actin that contract and relax to regulate blood flow in capillaries [19, 25]. Recruitment and proliferation of pericytes are mediated in part by platelet-derived growth factor B (PDGF-B) on endothelial cells, which binds with the corresponding receptor (PDGFR-B) on pericytes [19, 25].

As mentioned, the NVU is comprised of pericytes, astrocytes, microglia, and neurons [17, 18]. Compared to the murine brain, humans have more widespread pericytes and astrocytic endfeet [6]. Astrocytes within the NVU enhance tight junction formation, modulate the expression and polarization of transporters, and induce BBB characteristics in endothelial cells through the secretion of glial-derived neurotrophic factor (GDNF), basic fibroblast growth factor (BFGF), and angiopoietin-1 (ANG-1) [19]. Transplantation studies of astrocytes into non-CNS vasculature induced BBB properties both *in vitro* and *in vivo* [26, 27]. An additional barrier arises from astrocytic foot processes through the connection of tight and gap junctions [17, 28]. Astrocytes relay signals from neurons that regulate blood flow, including regulating the smooth muscle tone and contraction of pericytes [29]. For example, when microglia are activated by infection or injury, reactive oxygen species are released that can increase vascular permeability [29]. Additionally, microglia release growth factors that stimulate the development of new blood vessels and regulate the permeability of vasculature through the production of IL1β [30].

At least five instances are known of molecules passing through the BBB, including transcellular, carrier-mediated, paracellular, receptor-mediated, and adsorptive mechanisms [19, 31]. Lipid-soluble molecules smaller than 500 Daltons (Da) can diffuse across the membrane through a transcellular mechanism [19, 31]. Amino acids (*e.g.*, through Lat1), nucleosides, and glucose (*e.g.*, through Glut1) cross the BBB through a carrier-mediated mechanism [19, 31]. Some small hydrophilic molecules can be transported via a paracellular route [19, 31]. Larger molecules, including transferrin, ceruloplasmin, insulin, and albumin, cross the BBB through receptor-mediated processes with their unique receptors [19, 31]. Occasional large proteins such as albumin cross the BBB via adsorptive-mediated endocytosis/transcytosis. Many of these transporters and receptors are relatively enriched in the endothelial cells of the BBB compared to those of the kidney, lung, and liver [32]. Despite these existing BBB transport mechanisms, 98% of FDA-approved drugs do not penetrate the CNS [3, 33, 34]. Nearly all molecules greater than 500 Da will not cross the BBB [17]. Many lipid-soluble molecules, including drugs that cross the BBB through a transcellular mechanism, are removed from the CNS and sent back into the blood circulation through ATP-binding cassette transporters (ABC transporters), including multidrug-resistant (MDR) ABC transporters, multidrug resistance proteins (MRPs), P-glycoprotein (P-gp, ABCB1), and breast cancer resistance protein (BCRP, ABCG2) [17, 31].

2.2. Modulation of the Blood-Brain Barrier for Increased Drug Delivery

Improvements in treating CNS infections, amyotrophic lateral sclerosis (ALS), Alzheimer's disease, and other neurologic illnesses have been inhibited by failure to achieve therapeutic drug concentrations within the CNS [1, 5, 35]. The BBB also poses a challenge to obtaining adequate delivery of drugs in the treatment of CNS malignancies [9]. Glioblastoma, the most common primary brain cancer in adults, has maintained median survival of only 1-2 years for the past 20 years despite the accrual of thousands of patients on clinical trials [2]. Although cancer patients generally have seen increased survival due to improvements in therapy over recent years, brain metastasis has become more common and is often the first site of relapse due to low CNS penetration of tumor-directed medication [3].

There have been extensive efforts made to open the BBB and facilitate improved CNS drug penetration. These have included clinical studies of convection-enhanced delivery, intrathecal drug administration, focused ultrasound with microbubbles, and intraarterial mannitol [9-11]. Each approach has potential limitations and toxicities, but none are ideal for CNS diseases such as neurogenerative diseases, primary brain cancers, and brain metastases that are not localized to one region of the brain [5, 36-40]. Mannitol's ability to open the BBB with intraarterial administration has been studied for decades. This agent is administered intraarterially, requiring multiple inpatient admissions and invasive procedures, which can be associated with vascular complications. In addition, it opens the BBB only in the vascular distribution in which it is injected [11, 37, 38, 41].

Preclinical data indicate that the use of VAPs is a promising but understudied approach to transiently opening the BBB for enhanced drug delivery [12, 15, 39]. The bradykinin B2 receptor agonist labradimil (RMP-7, Cereport) has been shown to transiently increase BBB permeability and increase carboplatin delivery [40, 42-44]. More recent investigations into the A2A adenosine receptor signaling pathway

Table 1. Evidence classification of increased blood-brain barrier with vasoactive peptides.

Class 1	**Increased BBB permeability measured (imaging, tracers, PK studies)**
A	Evidence in humans
B	Evidence in laboratory mammals
C	Evidence *in vitro* model systems (*e.g.*, organoid, tissue-engineered BBB models, *etc*.)
Class 2	**Vasodilation of CNS vessels**
A	Evidence in humans
B	Evidence in laboratory mammals
C	Evidence *in vitro* model systems (*e.g.*, organoid, tissue-engineered BBB models, etc.)
Class 3	**Permeability in non-CNS vessels (*i.e.*, no BBB)**
A	Evidence in humans
B	Evidence in laboratory mammals
C	Evidence *in vitro* model systems (*e.g.*, organoid, tissue-engineered BBB models, *etc*.)
Class 4	**Biologic changes that support the opening of BBB**
	(*e.g.*, downregulation of tight junctions, inhibition of efflux pumps, *etc*.)

have shown promise in BBB disruption and CNS drug delivery [45-47]. The adenosine receptor agonist regadenoson, which is FDA-approved for cardiac stress testing, was found to increase brain levels of the chemotherapy temozolomide in rats by 60% without an effect on plasma concentrations [15]. An appealing feature of regadenoson induced BBB disruption is its very temporary effect on the BBB, as the persistent opening of the BBB could lead to significant cerebral edema [4, 15, 48].

This review was conceived based on the importance of delivering therapeutic doses of drugs to the CNS and the aforementioned studies of the BBB. Given that three VAPs have been shown to effectively disrupt the BBB in rodents, we sought to determine if there were other VAPs that deserved further preclinical evaluation.

3. METHODS

3.1. Literature Review

A narrative review of the literature was conducted using the National Library of Medicine (PubMed) and Google Scholar Databases using BBB and vascular physiology search terms with no restrictions on the date range. Studies in humans, animals, and *in vitro* experiments were included. A literature search was performed introducing these terms in various permutations: VAPs, BBB, permeability, vascular, vasodilation, endothelium, smooth muscle, pericytes, efflux pumps, tight junctions, and adherens junctions. The Food and Drug Administration's (FDA) approval status of these agents was identified. Manuscripts were limited to peer-reviewed articles published in English. No articles that met the above criteria were excluded.

3.2. Classification of Evidence and Recommendation Levels

To evaluate the likelihood of VAPs transiently disrupting the BBB, we devised a classification system to organize data from the literature review. In total, we designed four classifications based on the highest level of evidence in the literature for potentially opening the BBB in humans for each given compound (Table **1**). Class 1 compounds have been shown in the literature to increase BBB permeability as measured by imaging, pharmacokinetics (PK) studies, or tracers. Class 2 compounds are required to demonstrate clear evidence that they promote vasodilation of CNS vessels. Class 3 compounds do not meet the requirements for classes 1 or 2 but have evidence that they increase the permeability of non-CNS (systemic) vessels. Classes 1-3 have three subcategories: 1) evidence in humans, 2) evidence in laboratory animals, and 3) evidence from *in vitro* studies. Class 4 VAPs do not meet any of the criteria in classes 1-3 but do have studies indicating that they induce potentially favorable physiologic changes to open the BBB, such as downregulation of tight junctions, inhibition of efflux pumps, or modulation of the endothelium, smooth muscle, and pericytes.

4. RESULTS

We identified 32 VAPs with potential BBB permeability-increasing properties derived from 42 published articles using the criteria described above. We sorted them in a four-tier ranking system based on evidence in the literature of their ability to open the BBB to tracers with different physical properties (Table **2**) and physiologic properties that support the opening of the BBB (Table **3**). The highest level of evidence was used to classify the VAPs (Table **1**). This tiered system was created to provide guidance for the prioritization of VAPs and further study of opening the BBB.

4.1. Class 1 Vasoactive Peptides – Demonstration of Increased BBB Permeability

VAPs with class 1 evidence predominately derived data from the use of various tracers established to measure BBB permeability (Table **2**) [49-54]. As mentioned previously,

nearly all molecules greater than 500 Da will not cross the BBB, [17] and lipid-soluble molecules smaller than 500 Da can diffuse across the membrane through a transcellular mechanism [19, 31]. A wide variety of tracers with molecular weights ranging from 286 to 2,000,000 Da are used to assess BBB permeability in various experimental conditions [49, 50, 55].

Table 2. Selected tracers used to measure BBB permeability.

Marker	Size (Da)	Visualization
Radiolabeled sucrose	342	IL
Sodium fluorescein	376	LM
Lucifer Yellow	452	LM
Gadolinium contrast	550-800	MRI
TMR (dextran)	3000	LM, EM
Texas Red (dextran)	3000	LM, EM
Radiolabeled inulin	5000	IL
Evans Blue bound to albumin	69,000	Macro, LM
Dextrans (variety)	1500 -2,000,000	LM, EM

4.1.1. Class 1A Vasoactive Peptides – Evidence of Increased BBB Permeability in Humans

At this time, no VAPs have been conclusively shown to open the BBB in humans. Two agents have been studied in human trials with this goal as the primary endpoint. The first was a bradykinin agonist, labradimil, which had been shown to improve the delivery of carboplatin to the CNS and improve survival in glioma-bearing rats [39]. These findings led to a prospective phase 2 study of labradimil and carboplatin administered concurrently in patients with high-grade gliomas. Unfortunately, the endpoints were radiographic responses, and the study did not assess any possible increase in drug delivery to the brain [56, 57]. Because there was no clinical benefit to these treatments, which could either be from a lack of efficacy of carboplatin on targets or a lack of drug delivery, this line of research was abandoned and there have been no further investigations of labradimil opening the BBB. The second VAP studied in patients was regadenoson, an adenosine receptor agonist [45, 47, 58]. In rodents, regadenoson was found to transiently disrupt the BBB, allowing entry of a 70 kD dextran, disabling p-glycoprotein pumps, and facilitating the entry of lymphocytes into the brain. Regadenoson is FDA approved for patients who need a cardiac stress test but cannot walk on a treadmill [59]. There have been three pilot studies evaluating whether regadenoson administered at the FDA-approved dose for cardiac stress tests is able to open the BBB in patients. In the first study, patients undergoing clinically indicated regadenoson cardiac stress tests permitted their brains to be imaged after the cardiac stress tests were completed. The imaging agents used to assess the status of the cardiac vessels are usually excluded from the brain by the BBB. In these patients, there was no evidence that regadenoson administered at the FDA-approved dose for cardiac imaging increased BBB permeability of 99mTc-sestamibi, using SPECT imaging, or iodixanol (visipaque), using CT imaging [60]. A second study was carried out using FDA-approved doses of regadenoson in patients with high-grade gliomas undergoing a clinically indicated surgical debulking of the tumor. The primary endpoint was a $\geq$ 50% increase in temozolomide within brain interstitium concentrations after regadenoson. These patients consented to microdialysis catheters placed at the time of surgery. The following day in the ICU, they were given one dose of oral temozolomide, and a day later, they were given regadenoson and temozolomide. Intraparenchymal temozolomide concentrations were measured with and without regadenoson [61]. There were five patients who provided a collection of blood and dialysate samples. There were no significant differences found in blood levels of temozolomide between groups or in brain concentrations of temozolomide. The brain:plasma AUC ratios were 18.0 ± 7.8 and 19.1 ± 10.7% for temozolomide alone and with regadenoson, respectively. Animal studies have shown that mice and rats each require different concentrations of doses of regadenoson per body weight. It is likely that the FDA-approved doses of regadenoson, which were developed based on cardiac stress test criteria, are not the optimal doses to permeate the BBB in humans. As a result, there is now a multi-institutional NIH-funded clinical trial (NCT03971734) evaluating regadenoson to determine the optimal dose of this agent needed to open the BBB to MRI contrast (gadolinium). If a dose of regadenoson is identified that opens the BBB to gadolinium, then further studies will be designed to document that the entry of therapeutic agents into brain parenchyma also increased.

4.1.2. Class 1B Vasoactive Peptides - Evidence of Increased BBB Permeability in Animals

Eleven VAPs met the criteria for class 1B by demonstrating increased BBB permeability in animals. These studies utilized various tracers to evaluate the permeability of the BBB, including dextran, Evans blue, horseradish peroxidase, sodium fluorescein, and radiolabeled inulin. There are currently no imaging-based studies demonstrating VAPs increase BBB permeability in animals. These VAPs included adenosine, [46, 47] regadenoson, [15, 60] labradimil, [40, 42-44] bradykinin, [62] retro-inverso bradykinin (RI-BK), [63] endothelin-1 (ET-1), [64] C-type natriuretic peptide (CNP), [65] substance P, [66] angiopoietin-2, [51] interleukin-1β (IL-1β), [67] and captopril [68]. All VAPs were delivered intraarterially, except for adenosine, regadenoson, and CNP, which were administered intravenously, and IL-1β, and ET-1, which were administered intracranially in laboratory animals.

Both regadenoson and adenosine are ligands for the adenosine 2A receptor (A2AR), which belong to the family of G protein-coupled receptors and have been used clinically in cardiac stress testing [15, 60, 69, 70]. Adenosine has an active duration of 30-40 seconds, while regadenoson has a duration of 2-5 minutes [71]. These agents enhance nitric oxide (NO) production by smooth muscle and vascular endothelial cells [72, 73]. Increased BBB permeability by A2AR activation is mediated by cAMP/RhoA signaling, leading to downregulation of junctional proteins, including vascular endothelial cadherin (VE-cadherin), claudin-5, and reorganization of cytoskeletal actin [45, 46]. As mentioned previously,

Table 3. Vasoactive peptide rankings of evidence to potentially support opening of the blood-brain barrier.

Vasoactive Peptide	Level of Evidence	Increased BBB Permeability Marker	Vasodilation of CNS Vessels	Increased CNS Blood Perfusion	Increased Systemic Vessel Permeability	Vasodilation of Systemic Vessels	Smooth Muscle Relaxation	Modulate Endothelium	Down Regulate Tight Junctions	Increased Vesicular Transport	Block Efflux Pumps	Transient BBB Opening	Route Drug given and Animal Model	*In vivo* Dosing Used in Studies	FDA Approval
***NONE ***	1A	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Adenosine	1B	DX	X	X	X	X	X	X	X	X	X	X	IV, mouse	0.414 mg/kg	X
Captopril	1B	EB	X	X	-	X	X	-	-	-	-	-	IART, rat	10 mg/kg	X
Regadenoson	1B	EB	X	X	-	X	X	X	X	X	X	X	IV, rat; IV, mouse	0.0005 mg/kg; 0.05 mg/kg	X
Labradimil (RMP-7, Cereport)	1B	HP, EB	-	X	X	X	X	X	X	X	-	X	IART, rat	4.5 µg/kg	-
Bradykinin	1B	EB, HRP	-	X	X	X	X	X	X	X	X	-	IART, rat	10 µg/kg/min	-
Retro-inverso bradykinin (RI-BK)	1B	EB	-	X	X	X	X	X	X	X	X	-	IART, rat	30 µg/kg	-
Endothelin-1 (ET-1)	1B	SF	X	-	X	-	-	X	-	-	X	-	ICSF, canine	40 pmol	-
C-type natriuretic peptide (CNP)	1B	SF	X	-	X	X	X	-	X	-	-	-	IV, mouse	10 nmol/kg	-
Substance P (EP)	1B	HP	X	X	X	X	X	X	X	-	-	-	n/a**	n/a**	-
Interleukin-1β (IL-1β)	1B	HP	-	-	X	X	X	X	X	-	-	-	IBRN, rat	1 ng	-
Angiopoietin-2 (Ang-2)	1B	DX	-	-	-	-	-	-	X	X	-	-	n/a**	n/a**	-
Tumor necrosis factor-α (TNFα)	1C	IN	-	-	X	X	X	X	X	-	-	-	-	-	-
Calcitonin gene-related peptide (CGRP)	2A	-	X	X	-	X	X	-	-	-	-	-	IV, human	2 ug/min (mean 69.7 kg)	-
Urotensin II (U-II)	2B	-	X	-	X	-	-	X	-	-	-	-	ICSF, rat	1, 5, 10 nmol	-
Adrenomedullin	2B	-	X	X	-	X	X	-	-	-	-	-	IV, rat	0.1-1.0 µg /kg/ min	-

(Table 3) contd….

Vasoactive Peptide	Level of Evidence	Increased BBB Permeability Marker	Vasodilation of CNS Vessels	Increased CNS Blood Perfusion	Increased Systemic Vessel Permeability	Vasodilation of Systemic Vessels	Smooth Muscle Relaxation	Modulate Endothelium	Down Regulate Tight Junctions	Increased Vesicular Transport	Block Efflux Pumps	Transient BBB Opening	Route Drug given and Animal Model	*In vivo* Dosing Used in Studies	FDA Approval
Vasopressin (VP)	2B	-	X	X	-	X	X	-	-	-	-	-	IART, canine	100 pmol, 1 nmol	X
Oxytocin (OT)	2B	-	X	X	-	X	X	-	-	-	-	-	IART, canine	1 nmol, 10 nmol	X
Vasoactive intestinal polypeptide (VIP)	2B	-	X	X	-	X	X	-	-	-	-	-	IART, rabbit	10 μg/min	-
***NONE ***	2C	-	-	-	-	-	-	-	-	-	-	-	-	-	-
***NONE ***	3A	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Neurotensin (NT)	3B	-	-	-	X	X	X	X	-	-	-	-	skin, mouse	100 nmol	-
Corticotropin releasing hormone (CRH)	3B	-	-	-	X	X	-	-	-	-	-	-	skin, mouse	100 nmol	-
Interferon-α (IFN-α)	3C	-	-	-	X	-	X	-	X	-	-	-	-	-	X
Neuropeptide Y (NPY)	3C	-	-	-	X	-	-	X		-	-	-	-	-	-
Interleukin-2 (IL-2)	3C	-	-	-	X	X	X	-	X	-	-	-	-	-	X
Interferon-γ (IFN-γ)	3C	-	-	-	X	-	-	-	X	-	-	-	-	-	-
Phyllocaerulein	4	-	-	-	-	X	-	-	-	-	-	-	-	-	-
Phyllokinin	4	-	-	-	-	X	-	-	-	-	-	-	-	-	-
Phyllomedusin	4	-	-	-	-	X	-	-	-	-	-	-	-		-
Maxadilan	4	-	-	-	-	X	-	X	-	-	-	-	-		-
Sauvagine	4	-	-	-	-	X	-	X	-	-	-	-	-	-	-
Pituitary adenylate cyclase-AP	4	-	-	-	-	X	-	-	-	-	-	-	-	-	-
Urotensin-I (U-I)	4	-	-	-	-	X	X	-	-	-	-	-	-	-	-
Abaloparatide	4	-	-	-	-	X	X	-	-	-	-	-	IV, canine	0.1 μg/kg	X

Legend: DX= Dextran, EB = Evans blue, HP = Horseradish peroxidase, SF = Sodium fluorescein, IN = Inulin, IV = Intravenous administration, IART = Intraarterial administration, IBRN = Brain parenchyma administration, ICSF = cerebrospinal fluid administration through intracranial ventricle or cistern, ** = intracranial stroke physiology experiments in rodents with no drug given.

preclinical investigations found that the adenosine receptor pathway could open the BBB and could allow retention of drugs within the brain tissue by inducing a decrease in the expression of the drug efflux transporter P-glycoprotein and breast cancer resistance protein 1 (BCRP1) in a time-dependent and reversible manner [45, 47, 58]. Interestingly, A2AR expression is higher in human glioma margins, making this class attractive to increase drug delivery in these patients [74]. Although regadenoson was able to increase delivery of the chemotherapy temozolomide in the brain in rodents by transiently opening the BBB, it has not done so in humans at FDA-approved doses [15, 60, 61].

Captopril was evaluated based on studies showing significant amounts of angiotensin-converting enzyme in the microvessels of the brain. One study showed that intracarotid and topical application on the cerebral cortex led to an increase in regional cerebral blood flow and extravasation of Evans Blue [68]. It was believed that an increase in cerebral blood flow might impair local autoregulation associated with a marked rise in vesicular transport of tracer substances, leading to increased BBB permeability. The study noted an increase in PaCO2 with a decrease in blood pressure which may have contributed to vasodilation. Since captopril is a stimulator of prostaglandins release, which is vasodilatory, [75] some mice were pretreated with indomethacin which blunted the effects of captopril to increase BBB permeability. Kinins stimulate the release of prostaglandins, and the use of the kinin inhibitor aprotinin prevented the effects of captopril on increasing BBB permeability [68].

Bradykinin B2 receptor agonists such as bradykinin, retro-inverso bradykinin (a manufactured bradykinin analogue), and labradimil induce BBB permeability through induction of vascular transcytosis, TJ loosening, ATP-sensitive potassium (KATP) channels, Ca^{2+} flux, nitric oxide synthase (NOS), NO release (in part from increased intracellular Ca2+), cGMP and increases the number of pinocytotic vesicles and depolymerization of F-actin [12, 62, 68, 76, 77]. In brain tumor models of rats, bradykinin induces an accelerated formation of transendothelial pinocytotic vesicles in tumor capillary endothelium without affecting endothelial tight junctions. Additional studies demonstrate down-regulating the expression levels of tight junction proteins, ZO-1, occludin, claudin-5, and rearranging actin cytoskeleton [78, 79]. Interestingly, intracarotid bradykinin infusion increases the permeability of horseradish peroxidase and Evans Blue in glioma transplanted rats at the blood-tumor barrier (BTB) but not in regions of the brain with a normal BBB [79]. This effect is transient and lasts 20 minutes after cessation of the infusion [79].

Labradimil has been shown to increase the delivery of carboplatin into the brain of rodents [40, 42-44]. Labradimil opens the BBB transiently, and restoration of the BBB occurs within 2 to 5 minutes following cessation of infusion. Even with continuous infusion of labradimil, spontaneous restoration of the BBB occurred within 10 to 20 minutes [80]. RI-BK has resistance to proteolysis and has a 40× higher binding affinity to B2 receptor compared to bradykinin [12]. Interestingly, these studies show that RI-BK selectively increased the accumulation of nanocarriers in rodent glioma rather than in normal brain tissue through selective increased permeability of the BTB.

Endothelins (ET) are peptides that regulate neurogenesis, blood flow and pressure, apoptosis, and immune modulation [81]. There are three isopeptides of endothelin (1-3) that act on two receptors, ETA and ETB [81]. The brain is significantly enriched in ET-1, which is produced by neurons, astrocytes, and glial cells [82]. ET regulates the sympathetic nervous system, including modulation of cerebral blood flow through vasoconstriction [83]. One study found that intracisternal injection of ET-1 in canines significantly increased fluorescein penetration in cerebral spinal fluid [64]. Another study showed that ET-1 can induce an increase in the permeability of human brain capillary endothelial cells through receptor-specific activation of protein kinase C and intracellular calcium mobilization [84]. A study of isolated rat brain capillaries found that ET-1 rapidly and reversibly decreased P-glycoprotein–mediated transport function as demonstrated by luminal accumulation of a fluorescent cyclosporin A derivative. The mechanism by which this happened was in part due to the binding of ET-1 to the ET_B receptor, nitric-oxide synthase (NOS), and protein kinase C (PKC) [85]. Although known for its vasoconstrictive properties, endothelin infused in the vertebral artery of canines did not affect the caliber of the vasculature [86].

The family of natriuretic peptides includes atrial natriuretic peptide (ANP), B-type natriuretic peptide (BNP), and C-type natriuretic peptide (CNP) which are known for their essential role in electrolyte and fluid regulation as well as regulation of vasculature tone [87]. In our literature review, we did not find evidence that ANP or BNP could produce physiologic changes to the BBB to augment permeability. ANP binds to the BBB in both *in vivo* and *in vitro* experiments without altering its tight junctions, [88-90] and BNP may support the integrity of vascular barriers through stimulation of the proliferation, adhesion, and migration of endothelial progenitor cells [91]. In contrast, CNP has been shown to modulate the permeability of the BBB [65]. Bohara *et al.* used CNP dosed at 10 nmol/kg intravenously in mice and found increased sodium fluorescein permeability within the brain. In the same study, the transendothelial electrical resistance (TEER) of bovine brain microvascular endothelial cells and astrocytes dose-dependently decreased with CNP administration, indicating increased permeability. These investigations proposed that CNP modulates the BBB permeability by altering the expression of zonula occludens-1 (ZO-1), which plays a vital role in developing and stabilizing tight junctions by binding occludin to the cytoarchitecture [65, 92]. CNP, but not ANP or BNP, has vasodilating properties of the CNS, as demonstrated in rat cerebral arterioles through a cyclic guanosine monophosphate-dependent mechanism [93].

A review of the literature found that substance P (SP) plays a notable role in increased permeability of the BBB in brain ischemia [66]. SP can induce glutamate-mediated hyperexcitability, neurotoxicity, and can act on endothelial NK1 receptors, leading to venular permeability [66]. Additionally, SP promotes BBB breaching by breast cancer cells through changes in microvascular endothelial cell tight junctions, as demonstrated *in vitro* and *in vivo* in mice [94]. In this study, human brain microvascular endothelial cells were activated by SP, leading to the release of Tumor Necrosis Factor-alpha (TNF-α) and angiopoietin-2 (Ang-2), which

induced permeability through downregulation of ZO-1 and claudin-5 [94]. In another study, intravenous administration in 16 healthy male volunteers was found to increase blood flow and vasodilation of their forearms, suggesting a possible role of vasodilation in the CNS [95] Notably, rats intravenously injected with SP had increased leakage of horseradish peroxidase from dural vessels [96]. These studies suggest that substance P could open the BBB to improve drug delivery, although the potential limitation of SP is its role in maintaining status epilepticus [66].

Ang-2 has been shown to increase brain endothelial permeability *in vitro* and *in vivo* in rodents in one study using models of brain ischemia [51]. Interestingly, *in vivo*, Ang-2 increased permeability of Lucifer Yellow and 3 kD dextrans (Texas Red and tetramethylrhodamine) but did not increase permeability to the point of the passage of Evans blue dye, which is approximately 70 kD. The study found that Ang-2 increased BBB permeability through transcellular and paracellular mechanisms, including defective intra-endothelial junctions, increased vesicles, and degraded glycocalyx [51, 97].

IL-1β is has been found to induce BBB disruption by downregulating Sonic Hedgehog (SHH) in astrocytes. SHH induces upregulation of tight junction proteins resulting in increased integrity of the BBB byways of inactivation of protein patched homolog 1 (PTCH-1), which allows smoothened (SMO-1) to activate glioma-associated oncogene (GLI1), leading to upregulation of tight junctions [67]. IL-1β also increases astrocytic production of proinflammatory chemokines such as CCL2, CCL20, and CXCL2, inducing immune cell migration and exacerbating BBB disruption and neuroinflammation [67]. Increased BBB permeability was seen in mouse brain capillary endothelial cell lines using fluorescein isothiocyanate–labeled bovine serum albumin. Notably, injection of IL-1β into the striatum of juvenile rat brains results in a neutrophil-dependent increase in vessel permeability at 4 hours, as evident by extravasation of horseradish peroxidase with a similar study showing decreased apparent diffusion coefficient (ADC) MRI imaging of rats [98, 99].

4.1.3. Class 1C Vasoactive Peptides - Evidence of Increased BBB Permeability In Vitro

Tumor necrosis factor-α (TNFα) was the only class 1C compound and showed increased BBB permeability by directly affecting the endothelium [52]. IL-1β also is an indirect contributor to the increase in BBB permeability of tumor necrosis factor-α (TNFα) [52]. As described earlier, human brain microvascular endothelial cells can be activated by SP, leading to the release of Tumor Necrosis Factor-alpha (TNF-α) and angiopoietin-2 (Ang-2), inducing permeability through downregulation of ZO-1 and claudin-5 [94]. On monolayer endothelium, TNFα leads to increased TEER values, indicating increased permeability with an associated reduction in the tight junction proteins occludin and claudin-5 [100]. These effects were found to be ameliorated with concurrent hydrocortisone [100]. In bovine brain capillary endothelial cells, TNF-α notably did not have increased permeability with initial exposure but did so with rechallenge at 16 hours to radiolabeled sucrose (342 Da) and inulin (5 kDa) [52].

4.2. Class 2 Vasoactive Peptides – Demonstration of Vasodilation of Cerebral Vasculature

Generally, vasodilation within both the brain and systemic organs will result in increased vascular permeability. As a result, this could potentially serve as an indirect marker of agents capable of increasing BBB permeability [101, 102].

4.2.1. Class 2A Vasoactive Peptides - Evidence of Cerebral Vasodilation in Humans

Intravenous administration of calcitonin gene-related peptide (CGRP) has been shown to increase cerebral blood flow in patients with subarachnoid hemorrhage (SAH). It has also been found at high levels of blood in the jugular vein during migraine headaches [103, 104]. CGRP is a potent vasodilator that has physiologic roles in pain pathways (*e.g.*, migraines), wound healing, and the cardiovascular system [105]. CGRP can directly activate its receptors on vascular smooth muscle and mediate relaxation via the Gαs pathway, and activate receptors on endothelial cells to enhance NO production, leading to vasodilation through vascular smooth muscle guanylyl cyclase activation.[105] There are two protein isoforms, α-CGRP, and β-CGRP, which have similar physiologic roles, but α-CGRP is much more enriched in the central and peripheral nervous systems [106]. One study evaluated CGRP from venous blood of the antecubital fossa and external jugular vein of volunteers having migraines and found elevated levels of CGRP from blood in the external jugular vein only [103]. Another study in SAH patients found that administration of α-CGRP prevented SAH-associated vasoconstriction [104]. A double-blind, cross-over study in volunteer migraine patients administered α-CGRP and found a significant reduction of blood velocity in the MCA. These findings showed vasodilation but did not see a change in regional cerebral blood flow (rCBF) measured by Xenon-133 inhalation SPECT, indicating that there was no vasodilation of arterioles [107]. CGRP may not be an ideal agent to increase drug delivery through the BBB given its properties in strengthening it by upregulation of growth factor signals from the endothelium to the brain parenchyma and due to its protection of the immune privilege in the brain through various mechanisms, including preservation of tight junctions [108].

4.2.2. Class 2B Vasoactive Peptides – Evidence of Cerebral Vasodilation in Animals

There were five VAPs that met group 2B criteria of cerebral dilation without the ability to open the BBB. This includes adrenomedullin [109-111] and urotensin-II (U-II), which demonstrated vasodilation of CNS vessels in rats with intraventricular but not intravenous administration, [112] intraarterial vasopressin, oxytocin, and vasoactive intestinal peptide (VIP) in mammals [86, 113].

Adrenomedullin is a vasoactive peptide with structural homology to CGRP that has shown an increase in cerebral vasodilation topically, intraarterially, and intravenously in mammals and is primarily mediated by activation of $CGRP_1$ receptors with dependency on activation of K^+ channels [109-111].

Urotensin-II (U-II) is an endogenous peptide within the cardiovascular system that plays a role in the contraction of vascular smooth muscle and endothelial-dependent vasorelaxation through binding of Gαq-coupled receptors and has

been found to induce endothelial hyperpermeability on experiments using rat aorta tissue [114]. Whether U-II results in vasoconstriction or vasodilation depends based on the vascular bed in question and differs between species [112]. In one experiment, rats intravenously treated with U-II had no change in cerebral blood flow (CBF). However, intraventricular injection of U-II leads to hyperperfusion in the ischemic brain compared to the control in rats with occlusion of their middle cerebral artery [112]. The precise mechanism from this study was not clear but is hypothesized to be related to pathways involving NO production. Normal brain tissue was not reported to be affected in the study either grossly or on histologic analysis [112]. Another study found that topical U-II on pial arteries of newborn pigs visualized with microscopy through a closed cranial window had vasodilation. They further determined that vasodilation occurred in a NO-dependent manner [115].

Vasopressin (VP) and oxytocin (OT) complement each other with VP resulting in water retention and OT facilitating the removal of sodium [116]. In most vascular beds, VP and OT are vasoconstrictive, but in the brain and lungs, they cause vasodilation in a NO-dependent manner based on an experiment using canine basilar contracted with prostaglandin F2 alpha [116, 117]. In another study, canines infused with VP and OT via the vertebral artery had dilation of the vertebral, anterior spinal, basilar arteries, and the circle of Willis and its branches [86]. Interestingly, vasopressin may modulate the BBB to increase permeability in autoimmune encephalomyelitis and blockade of arginine vasopressin receptors prevents BBB breakdown [118].

VIP is a vasodilating peptide found within the central and peripheral nervous systems [119]. In one study, intracarotid VIP in rabbits increased blood flow selectively to cerebral gray matter but not white matter [113]. Conversely, another study in which the common carotid artery of canines was infused with VIP which led to significant dose-dependent increases in the common carotid artery and temporalis muscle, but there were no effects on total or regional cerebral blood flow [120].

4.2.3. Class 2C Vasoactive Peptides - Evidence of Cerebral Vasodilation In Vitro

No VAPs were found that met the criteria for class 2C level of evidence.

4.3. Class 3 Vasoactive Peptides – Demonstration of Increased Permeability of Peripheral Vasculature

Systemic vasculature shares some common features with the BBB, making evidence of increased non-CNS vasculature permeability by VAPs an indirect and favorable attribute to explore further [6, 102].

4.3.1. Class 3A Vasoactive Peptides - Evidence of Increased Non-CNS Vascular Permeability in Humans

No VAPs were found that met the criteria for class 3A level of evidence.

4.3.2. Class 3B Vasoactive Peptides - Evidence of Increased Non-CNS Vascular Permeability in Animals

Neurotensin (NT) and corticotropin-releasing hormone (CRH) were classified as class 3B through demonstration of increased skin vasculature permeability in mouse and rat skin [121]. In one study, intradermal NT and CRH in mice showed increased vascular permeability with Evans Blue extravasation [121]. Using knockout mice, they found that NT is necessary for CRH to cause vasodilation and that NT stimulates mast cells to release vasodilatory molecules including histamine, NO, tryptase, TNF-α, VIP, and vascular endothelial growth factor (VEGF).

4.3.3. Class 3C Vasoactive Peptides - Evidence of Increased Non-CNS Vascular Permeability *In Vitro*

Class 3C peptides have evidence of *in vitro* increase in permeability of system vessels and include interferon-α (IFN-α), [122] interferon-γ (IFN-γ), [122] neuropeptide Y (NPY), [123] and interleukin-2 (IL-2) [124].

IFN-α is FDA approved to treat various leukemias, lymphomas, melanoma, and Kaposi's sarcoma, and IFN-γ is associated with antiproliferative, pro-apoptotic, and antitumor mechanisms in cancer [125, 126] Studies on human dermal microvascular endothelial cells and fibroblasts from foreskins of healthy newborns showed that both IFN-α and IFN-γ could contribute to the increased vascular permeability through downregulation of friend leukemia integration 1 transcription factor (FLI1) and VE-cadherin [122].

NPY is a neurotransmitter that contributes to various physiologic processes, including cortical excitability, stress response, food intake, circadian rhythms, and cardiovascular function [127]. In one study, NPY increased permeability across a monolayer of rat aortic endothelial cells in hypoxic but not normoxic conditions [123]. The authors hypothesized that this finding is mediated by direct action on the NPY Y_3 receptor expressed on the endothelial cell membrane rather than due to NO, bradykinin, or VEGF. Another study found that infusions of NPY in the vertebral arteries of canines did not result in vasodilation of intracranial vasculature [86].

IL-2 is a cytokine that has a role in expanding T lymphocytes and natural killer cells and is FDA approved to treat renal cell carcinomas and melanoma [125]. Studies on human and murine brain microvascular endothelial cells showed that IL-2 induces increased permeability through induction of phosphorylation of VE-cadherin, which resulted in dissociation of supporting proteins, including catenins, and a reduction of Src homology 2 domain-containing protein-tyrosine phosphatase 2, which is known to support vascular barrier function [124].

4.4. Class 4 Vasoactive Peptides - Evidence of Biologic Changes that Support Opening of the BBB

Eight class 4 peptides were identified with literature suggesting favorable biologic changes to open the BBB, including phyllocaerulein, phyllokinin, and phyllomedusin, which are derived from the of the frog *Phyllomedusa bicolor*, [128] abaloparatide, [129, 130] maxadilan, which is derived from the sandfly *Lutzomyia longipalpis*, [131] sauvagine, [132], urotensin-1 (U-I), [133] and pituitary adenylate cyclase-activating polypeptide (PACAP) [134].

Kambô, also known as Sapo, is a ritual performed in some South American countries where the user inoculates an open wound with secretions from the frog *Phyllomedusa bicolor,* causing sedation, tachycardia, dizziness, nausea, and

vomiting [135]. Within these secretions are the VAPs phyllocaerulein, phyllokinin, and phyllomedusin, which have vasodilatory properties [128, 136].

Abaloparatide is a parathyroid hormone-related protein (PTHrP) analog that is FDA approved to treat postmenopausal women with osteoporosis at high risk for fracture [137]. PTHrP and associated receptors are found in vascular smooth muscle cells that appear to have a role in regulating vascular tone [129]. Vasodilation of rodent femoral principal nutrient arteries was found with the administration of parathyroid hormone (PTH) analogs, including PTHrP, and is believed to occur through activation of the endothelial cell PTH 1 receptor, (PTH1R) resulting in NO-mediated vasodilation [130]. Additionally, in the European Medicine Agency's assessment report of abaloparatide (EMA/CHMP/581111/2018), anesthetized dogs given intravenous boluses of abaloparatide had dose-dependent peripheral arteriolar vasodilatation as well as chronotropic and inotropic effects.

Maxadilan is a vasodilating peptide isolated from the salivary gland of the sand fly *Lutzomyia longipalpis* [131]. Vasodilation occurs via an endothelium-independent mechanism through the accumulation of intracellular cyclic adenosine monophosphate (cAMP), as demonstrated in isolated aortas of rabbits [138].

The corticotropin-releasing factor family plays a role within the stress-hormone receptor system that regulates vascular tone and includes CRF, sauvagine, and urotensin-1 [139]. In one study using endothelium-denuded rat thoracic aorta and rat superior mesenteric arterial vasculature, both CRF and sauvagine produced vasodilation lasting more than 20 minutes by prolonged activation of NO synthase [132]. In another study, urotensin I resulted in vasodilation in rat aorta, both in the presence and absence of endothelial cells [133].

PACAP has structural homology to vasoactive intestinal peptide and has diverse functions, including catecholamine secretion based on its prevalence in sympathetic neurons and the cholinergic innervation of the adrenal medulla [140]. Receptors for PACAP are found in vessel walls and infusion of PACAP causes vasodilation of the middle meningeal artery in both rats and humans [134].

5. DISCUSSION

The BBB presents a formidable challenge to improving outcomes for patients with a wide variety of brain diseases [1-3, 5, 35]. Effective treatment for many of these disorders will require that systemically administered agents reach the brain in therapeutic concentrations. One potential way to improve CNS drug delivery is to co-administer the therapeutic agent with another that transiently disrupts the BBB. VAPs represent a promising drug class for this purpose as they appear to have the capacity to open the BBB transiently to increase drug delivery in animals. Ideally, a candidate VAP would have robust supporting preclinical data documenting its ability to alter BBB permeability and have FDA approval for another purpose. Regadenoson is an example of such a candidate [15]. Most VAPs have not been evaluated on their ability to open the BBB. Prioritization for new VAPs to be developed for this purpose should include mechanisms of action, route of administration, and the degree and duration of BBB versus BTB opening. If this approach to increasing drug delivery to the CNS is successful, significant challenges will emerge as many patients are routinely taking FDA-approved, non-CNS penetrant drugs for other medical conditions which may be neurotoxic if these agents are suddenly granted easy access to the CNS.

A common pathway found in many VAPs appears to be nitric oxide (NO). NO is known for its potent vasodilatory effects, its relationship to injury-related vasogenic edema, and the potential to induce BBB permeability transiently [12]. NO, a free radical, is synthesized from L-arginine via nitric oxide synthase (NOS) [73]. NO, once synthesized, diffuses and activates soluble guanylyl cyclase converting GTP to cGMP, thus activating cGMP-dependent protein kinase, reducing intracellular calcium, releasing acetylcholine, and reducing vascular smooth muscle tone [141]. The mechanism of endogenous NO release has been attributed to endothelial stimulation by adenosine, bradykinin, tumor cells, and injury [12, 72]. Bradykinin, through bradykinin type 2 (B2) receptor agonism, increases NO production via stimulation of NOS inducing BBB permeability and has been shown to open the BTB in rodents with brain tumors [42, 62]. Similar to bradykinin, adenosine via its receptors A1 and A2 results in NO production directly and selectively in arterial endothelium and increased production and accumulation of NOS messenger RNA (mRNA) [72]. Chemical compounds formulated to release NO (named NO donors) have been shown to transiently induce BBB and BTB permeability with minimal effects on hemodynamics (e.g., significant changes in arterial blood pressure and cerebral or tumor perfusion).[142] NO donor mediated permeability originates from the same common pathway as bradykinin-induced NO release [142].

When evaluating viable VAPs, the region(s) of vascular modulation deserve consideration. As Lecrux *et al.* found, the effect of urotensin-II (U-II) on vascular beds varied based on organ-specific systems [112]. The dependent influence of U-II extended beyond organs and demonstrated variation based on species-specific vascular beds. Beyond vascular bed vasodilation variability, some prospective VAPs may show promising physiologic effects in systemic vessels but produce paradoxical effects in the cerebral vasculature. Such is the case for apelin, which vasodilates peripheral vasculature but inhibits NO-induced cerebral arterial relaxation. As Mughal *et al.* demonstrated, this is related to inhibition of large conductance, calcium-activated K channels in cerebral arterial smooth muscle cells [143]. Thus, it is essential to test and evaluate the effect of VAPs in brain vascular beds while considering ideal mammalian models. Importantly, an ideal VAP will have a demonstrable impact on vascular beds at the capillary level and healthy intact BBB rather than only within neoplasms or other disease tissue of the brain. This point is critical to consider in the context that some agents may only vasodilate large vessels without inducible permeability or effect on capillary level vasodilation. For example, in one study α-CGRP was administered to human volunteers and regional cerebral blood flow (rCBF) measured by Xenon-133 inhalation SPECT was unchanged despite MCA vasodilation [107]. An ideal prospective VAP would induce permeability throughout the entire BBB, thereby increasing

drug delivery for diffuse disease processes affecting the brain including radiographically undetectable micrometastases, gliomas, and degenerative CNS disorders such as Alzheimer's disease [1-3]. These desired traits make approaches such as intracarotid bradykinin less appealing, as they increase the permeability of Evans Blue in glioma transplanted rats at the BTB but not the BBB [79]. Furthermore, studies of drugs using transplanted gliomas may be misleading as these tumors often do not recapitulate a spontaneously occurring glioma which has a diffuse microscopic disease and a BTB with open and closed regions [144].

The method of VAP administration also has practical and therapeutic implications. Intravenous delivery would be the most logical, convenient, and cost-effective method of administering VAPs. Intraarterial administration has inherently higher risks, limited distribution within the CNS, and more potent effects on hemodynamics. In addition, the level of complexity increases if the tumor extends across multiple vascular beds requiring a series of infusions in different vessels. Direct intracerebral injections are invasive and require properly placed catheters for direct infusion or convection-enhanced delivery. Intranasal delivery of VAPs could be considered an alternate delivery approach once an attractive drug candidate is found [145]. While this approach is frequently used in rodents, there are notable anatomic differences between rodents and primates in their olfactory bulbs, olfactory tracts, trigeminal nerves, and surface areas for absorption of intranasal medications. The olfactory epithelium covers 50% of the nasal mucosa in rats and only 10% in humans [146]. These factors could limit the utility of intranasal drug administration in humans.

Limiting the opening of the BBB to a short period of time is another critical factor to consider. This barrier has been preserved and refined through millions of years of evolution which speaks to its physiologic importance [4, 48]. To facilitate drug entry, the BBB must be open long enough for a significant amount of the therapeutic agent to access the CNS. The delivery and timing of VAPs depend on multiple factors that must be considered unique in each situation. These factors include the rate of penetration of an intended therapeutic agent in the setting of a chosen VAP and the pharmacokinetics and pharmacodynamics of the VAP and therapeutic agent. Some VAPs may require boluses and others may need continuous infusions or a combination of the two for effectively inducing transient permeability.

Translation of VAPs into patient care must account for clinical factors such as medication and medical history. For example, bradykinin synergizes with L-arginine and hydroxyurea but is inhibited with the use of indomethacin, trifluoperazine, or imidazole [77]. Similarly, aminophylline and caffeine may impair the activity of adenosine agonists [147, 148]. Relative contraindications to the administration of VAPs will also need to be considered. For example, heart block, reactive airway disease, chronic obstructive pulmonary disease (COPD), and asthma would likely be contraindications for the use of adenosine [149]. Hypothyroidism, which augments aortic endothelial receptor response to adenosine, could also result in a more significant vasodilatory response than is expected in euthyroid patients [150]. In addition, VAP may have more than one mechanism of action. For example, both regadenoson and adenosine increase NO production but some studies suggest that NO can increase cancer progression and treatment resistance [151, 152].

The effect of VAPs in different animal species must also be recognized when deciding which VAPs should be moved into human trials. O'Brown *et al.* showed varying characteristics among species, including humans. Compared to the murine brain, humans have more widespread pericytes and astrocytic endfeet [6]. Additionally, adenosine modulated changes in cerebral vasculature have been documented in rabbits and baboons but not in dogs or cats [153]. When evaluating VAPs for their effectiveness in transiently inducing BBB permeability, assessment in human models is ideal for study as the anatomical differences among model species and humans may contribute to variation in the effectiveness of VAPs. Molecular target receptor expression in models and humans can impact the effect of VAPs on BBB. The verification and cataloging of available receptors for targets in various models can provide valuable information for VAP selection. For example, adora2a, an adenosine analog, is minimally expressed in mouse brain endothelium while abundant in many other mural cell populations. The use of molecular atlases, such as those aggregated by Vanlandewijck *et al.* in 2018, could provide valuable information for understanding the viability of select VAPs dependent on expressed target receptors [154].

This literature review revealed that some VAPs might be of utility for inducing BBB permeability; however, there are many others with unknown vasoactive potential and even more that have yet to be evaluated in the brain and the BBB. The majority of VAP investigations have focused on the ability of single agents to induce transient permeability in cerebral vessels. Future research on combinations of VAPs with different mechanisms of action on cerebral vessels is needed. For example, a combination of VAPs that inhibit drug efflux pumps, transiently vasodilate, and transiently relax tight junctions may be more effective than a single agent. The complexity of the BBB offers multiple targets for inducing BBB permeability. For instance, IL-1β stimulation of microglia and peripheral immune cells might well lead to increased BBB permeability [17]. These ancillary cells may be an accessory pathway for inducing BBB permeability and may provide additional synergistic effects with target receptor stimulation.

CONCLUSION

Allowing medications to enter the brain in therapeutic concentrations is of significant importance to improving the outcome of patients with neurologic disorders. Several VAPs show promise in transiently opening the BBB in animals, and one is currently being studied to determine if it has efficacy in humans. As summarized in this manuscript, there are many other VAPs with the potential to transiently disrupt the BBB which have not been evaluated preclinically for this purpose. These compounds should be assessed thoroughly as single agents and in combinations using *in vitro*, animal, and human studies to determine if VAPs can improve CNS drug delivery.

AUTHORS' CONTRIBUTIONS

Conceptualization, writing, and editing were done by M. Smith-Cohn, N. Burley, and S. Grossman. All authors have read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST

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