The Role of Pericytes in Cerebral Blood Flow: Mini Review

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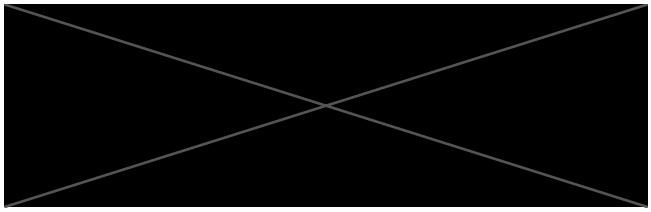

SUMMARY

For many years the role of capillary pericytes in cerebral blood flow has been debated. A literature search of the last 10 years for research on the subject was performed to evaluate the current evidence where a total of 8 articles were carefully selected. The findings showed expression of α-smooth muscle actin in pericytes along with changes in capillary diameter upon pericyte stimulation with different techniques.

1.0 BACKGROUND

1.1 Mechanisms of cerebral blood flow

It is classically well known that the energy expenditure required to maintain proper brain function is quite large compared to the brain's small percentage of total body mass. Therefore, consistent cerebral blood flow is extremely important for optimal brain health and must be tightly regulated by several mechanisms. Such mechanisms can be boiled down to two main processes which are autoregulation and neurovascular coupling (1). At the center of each of these mechanisms is the neurovascular unit (NVU) which is built specifically for efficient blood flow to facilitate proper exchange of gases, nutrients, and waste products as well as protection. The NVU is composed of neurons, glial cells, vascular smooth muscle, endothelial cells, and pericytes which make it possible to keep constant blood flow and redirect blood toward increased neuronal activity (2).

Autoregulation is an important mechanism for consistent cerebral blood flow because it responds to blood pressure changes to ensure that there are no changes in total cardiac output to the brain. Autoregulation is dependent on the myogenic response which is the response of smooth muscle cells of blood vessels to changes in arterial pressure by either constricting or dilating (1). The myogenic response is activated by stretch, G-coupled protein receptors, and Ca^{2+} increases which results in the contraction of smooth muscle cells in intracerebral arteries and arterioles ultimately causing vasoconstriction (1).

In contrast, neurovascular coupling is a more localized response at the capillary level to autoregulatory responses at the arteriole level integrated with responses to changes in neuronal activity (1). The blood flow in brain capillaries is tightly mediated by different compounds released by excitatory or inhibitory neurons such as neurotransmitters, ions, nitric oxide, and oxygen levels. Excitatory neurons typically induce sizeable increases in oxygen usage due to an overall increase in surrounding neuronal activity (1). As the activity of neurons fluctuates, the vasculature must adapt through several mechanisms to increase the blood flow to a specific area without reducing blood flow from nearby vascular networks. One such mechanism is the role of pericytes in neurovascular coupling with capillary blood flow which will be discussed further in the section below. The role of pericytes in cerebral blood flow has been overlooked in the past, but has gained traction in the literature in the last decade (2).

1.2 What are pericytes?

Pericytes can be described as small cells that are scattered on the surface of capillaries with projections that wrap around endothelial cells. These cells are critical pieces of the microcirculation environment and are vital for the development of blood vessels, blood-brain barrier (BBB) maintenance, and regulation of cerebral blood flow.

Blood Vessel Development and BBB Maintenance

During development, platelet-derived growth factor BB (PDGF-BB) is the most important molecular signal released by endothelial cells for facilitating pericyte recruitment and retainment (3). Loss of its receptor, PDGFRβ which is a receptor tyrosine kinase, can result in significant complication with vascular function. The transcription factor, Foxf2, is exclusively expressed by brain pericytes and has been implicated in their function in vascular maturation and BBB maintenance. One study showed that deletion of the Foxf2 gene led to an increased number of pericytes, but with heavily reduced expression of PDGFRβ and signalling (3). This eventually led to a steady increase in BBB permeability in inactivated Foxf2 mice and failure to develop a BBB in Foxf2 knockout mice (3).

Cerebral Blood Flow

In cerebral blood flow, the specific role of pericytes is currently debated in the literature, however, there is clear evidence to support their involvement. For example, it has been shown that reduction in PDGFRβ not only leads to BBB breakdown, but also reduces the efficiency of neurovascular coupling (1). One explanation for this has been the possibility that pericytes dilate or constrict in response to neurovascular coupling activity to vasodilate or vasoconstrict capillaries. A study by Hall et. al. 2014 imaged different orders of blood vessels following whisker pad stimulation to determine vasodilation initiation. They found that capillaries dilated before arterioles which suggests the role of pericytes in detecting changes in neuronal activity to initiate capillary dilation rather than pressure changes from arteriolar dilation (4). Another study collected evidence that indicates that pericytes express α-smooth muscle actin (α-SMA) which would explain their contractile abilities (5). However, this has been contradicted by other studies, for example Hill et. al. entirely refutes the idea that capillary pericytes in the brain express α-SMA at all (6). Other studies have found similar results that describe that precapillary arterioles vasodilate prior to capillary dilation which contradicts the results in Hall et. al. 2014 (1, 4). One explanation for this phenomenon could be due to different phenotypes of pericytes at certain locations of the neurovascular unit and on precapillary arterioles (1). In general, the subject of pericytes in cerebral blood flow regulation continues to be a subject of controversy in the field. Therefore, the purpose and aim of this review is to gather evidence from the current literature to explain the role of pericytes in cerebral blood flow and critically evaluate studies that support or oppose their role.

2.0 METHODS

2.1 Literature Search

The study was performed by following systematic review guidelines for a mini review of pericyte function in cerebral blood flow. The international database, Pubmed, was used to conduct a literature search of articles published over the last 10 years between 2013 and 2023. A total of 6 search terms/phrases were used to identify the most relevant original articles. The keywords/phrases included in the searches were pericytes, neurovascular, cerebral blood flow, α-smooth muscle actin, neurovascular coupling, and capillary. All stages and results of the selection process are summarized in Table 1.

2.2 Article Eligibility Criteria and Data Compilation

Articles included in this study were required to meet the following criteria:

1. Original research article.
2. All keywords or similar within each search term in the title.

3. Related to cerebral blood flow and pericyte contribution or lack thereof.
4. Published between 2013 and 2023.

Exceptions that fulfilled eligibility criteria but led to exclusion were:

1. If there was a focus on a specific peripheral condition such as diabetes.
2. If there was a greater focus on the spinal cord instead of the brain.
3. If main focus of the study was not to prove pericyte function in cerebral blood flow but it was relevant to the study's main focus.

3.0 RESULTS

3.1 8 Key Articles Identified in Pubmed

A total of 6 search phrases were entered into Pubmed to identify key articles describing the function of pericytes in cerebral blood flow. From the literature search, a total of 8 articles were selected that were published between 2013 and 2023, a free source, had key words in the title, and were original research articles. Of the selected articles, 7 of the 8 were repeated in at least 2 of the search phrases. A summary of the results is outlined in Table 1.

Table 1: Literature search. Outline of the literature search stages in Pubmed for key phrases and search terms for pericytes in neurovascular function. Each stage was utilized to narrow the most relevant articles. *In the final selection, some articles repeated within the search terms.

	Primary Database: Pubmed	**Stage 1**	**Stage 2**	**Stage 3**	**Stage 4**	**Final Selection***
Keywords	*Search volume*	*2013-2023*	*Free source*	*Title*	*Title* + *Original Article* + *Abstract*	Total: 8 (*Repeated articles)
Pericytes in neurovascular function	687 results	602 results	425 results	19 with pericytes + neurovascular	7 results	1 result*
Pericyte function in cerebral blood flow	395 results	272 results	193 results	13 with pericytes + "cerebral blood flow" or "brain blood flow"	9 results	6 results*
Capillary pericyte blood flow	727 results	331 results	231 results	10 results with pericytes + "capillaries" + "blood flow"	10 results	6 results*
Capillary pericytes and α-smooth muscle actin	137 results	50 results	27 results	3 results with pericytes + "α-smooth muscle actin" or "α-SMA"	3 results	1 result
Pericytes and neurovascular coupling	120 results	112 results	88 results	2 results with pericytes +	2 results	1 result*

				"neurovascular uncoupling"		
Brain pericyte contractility	104 results	75 results	54 results	9 results with pericytes + "contractility" or "contractile" or "contraction"	6 results	2 results*

Of the 8 selected articles:

1. 1 provides evidence that pericytes either do not contribute to cerebral blood flow or have a limited contribution.
2. 7 provide evidence that pericytes modulate capillary constriction and/or dilation in the brain.
    - 3 articles provide evidence that pericytes express α-SMA.
    - 3 articles show how pericytes impact blood flow during ischemia.
    - 1 article provided evidence that pericyte reduction can leads to neurovascular uncoupling and an overall reduction in oxygen consumption.
    - 2 articles show how calcium and potassium channels in pericytes contribute to modulation of cerebral capillary blood flow.

4.0 DISCUSSION

Presently, the role of pericytes in cerebral blood flow has been debated in the literature due to identifying contractile characteristics that would aid capillary blood flow. Therefore, the aim of this review was to identify key original research that investigated the role of pericytes in cerebral blood flow between 2013 and 2023 to make a conclusion about their function. After systematic review of 8 separate studies, the findings were that overall, the majority of recent evidence supports that pericytes do contribute to the regulation of cerebral blood flow.

4.1 Distinguishing Between Pericytes and Arteriolar Smooth Muscle Cells to Separate Capillaries and Arterioles

One of the major challenges that has caused controversy in the past about pericytes regulating cerebral blood flow has to do with the detection of contractile proteins, most notably, α-SMA, an important contractile actin expressed in smooth muscle cells. Some of the key studies identified in the literature have shown that pericytes also express α-SMA to a lesser extent than smooth muscle cells which would explain their possible contractile abilities. However, the study Hill et. al. 2015 did not confirm this to be the case both with in vivo 2-photon microscopy and in vitro immunohistochemistry stains on mouse brain slices (6). They also did not detect expression of α-SMA in human brain tissue staining. Additionally, while they observed significant changes in diameter at junctions between precapillary arterioles and capillaries using whisker pad stimulation and optogenetic stimulation, they did not observe any changes in diameter using these techniques in downstream capillaries. Interestingly, this finding leads to another issue in determining the role of pericytes in regulating cerebral blood flow which is clearly defining and characterizing the morphology of pericytes and smooth muscle cells to distinguish them and their locations.

While there does not appear to be disagreement on that arteries and arterioles express continuous layers of smooth muscle while capillaries do not and instead are covered in pericytes, there does seem to be confusion regarding the separation between pericytes and smooth muscle cells. In particular, one specific pericyte phenotype described in Gonzales et. al. 2020 (7). In the study they identified three categories of pericytes: arteriole proximate/ensheathing, mesh type, and thin strand which can be seen in Figure 1. Arteriole proximate pericytes were described as having short extensions, densely packed ensheathing capillaries, and similar in appearance to smooth muscle due to their dense banding pattern. However, they were distinguished from smooth muscle because of the pericyte projection wrapping pattern from a single cell. Mesh type pericytes were defined as having moderate capillary coverage, long extensions, and lack of dense banding as seen in the arteriole proximate pericytes. Finally, thin strand pericytes had long extensions, but fewer projections and minimal wrapping around capillaries. The difficulty with distinguishing pericytes and smooth muscle cells to define where the arteriole ends, and the capillary begins is that according to Gonzales et. al. 2020, the arteriole proximate pericytes can be confused for smooth muscle cells as they are tightly packed at the junction between the feeding arteriole and first order capillary. This region is therefore, sometimes termed as the “precapillary arteriole” and that the arteriole proximate pericytes are considered as atypical smooth muscle cells (7). By this definition, the findings of Hill et. al. 2015 would instead have confirmed pericytes as contractile and contributing to cerebral blood flow as their results identified significant changes in diameter at the precapillary arteriole as well as expression of α-SMA (6).

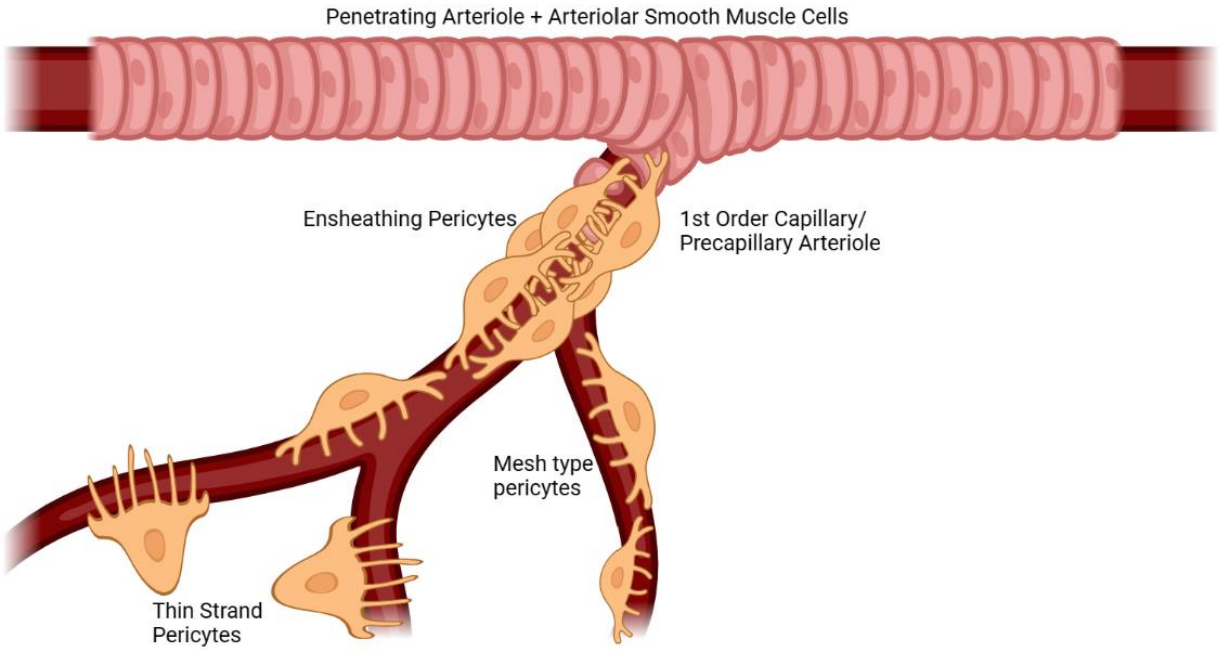

Figure 1: Categories of pericytes as described in Gonzales et. al. 2020 (7). The 1st order capillaries are densely packed and layered with pericytes that have short processes. Mesh type pericytes are moderately spread out with slightly longer processes. Finally thin strand pericytes are most scattered and have very long processes. This figure was created using Biorender.com.

In the study Hall et. al. 2014 they defined capillaries as being less than 10 μm in diameter and lacking smooth muscle (4). However, in Hill et. al. 2015 they define capillaries as 3 – 9 μm, precapillary arterioles as 3 – 15 μm, and penetrating arterioles as 15 – 40 μm in diameter (6). In both cases, there is a problem in that some of the results of these two studies could be concluded

differently depending on how they have distinguished between capillaries and arterioles. However, just as Gonzales et. al. 2020 described before about confusion surrounding the definition of a precapillary arteriole, Hall et. al. 2014 also comments on this notion that previous studies outside the scope of this review have also confused pericytes with smooth muscle cells (4, 7).

Overall, there needs to be more distinct criteria that defines a capillary and an arteriole apart from diameter, smooth muscle and pericytes to apply more consistency for whether a blood vessel should be termed as a first order capillary or a precapillary arteriole. Therefore, Gonzales et. al. 2020 used additional criteria to separate capillaries from arterioles (7). Not only do arterioles have smooth muscle cells, but they also have an internal elastic lamina, whereas capillaries do not. They were able to separate capillaries from arterioles by staining preferentially for internal elastic lamina with hydrazide and were then able to assume all downstream vessels were capillaries, in which all cells with projections surrounding them could be considered as pericytes (7). This helps to alleviate confusion when observing the morphology of pericytes and smooth muscle cells at the junction between feeding arterioles and first order capillaries because the common markers for pericytes, neural/glial antigen 2 (NG2) and PDGFRβ, are also expressed in arteriolar smooth muscle cells (6). Another study by Hartmann et. al. 2021 took a different approach in defining where pericytes are located by using the term precapillary arteriole but recognized that the ensheathing/arteriole proximate pericytes described in Gonzales et. al. 2020 were expressed there rather than smooth muscle (8). While this definition does not make complete sense since arterioles should contain smooth muscle, this further highlights the importance of having consensus on definitions, morphological criteria, and the ability to identify those criteria when performing an experimental study.

4.2 Evidence for Pericyte Regulation of Cerebral Blood Flow

Pericytes Express α-SMA

Returning to the debate about pericyte expression of α-SMA, even if Hill et. al. 2015 redefined precapillary arterioles as first order capillaries, they still did not detect expression of α-SMA in downstream pericytes. The study Alarcon-Martinez et. al. 2018 proposed that α-SMA may quickly depolymerize in pericytes during perfusion and fixation which would then explain the lack of detection when immunolabelling (5). However, Hill et. al. 2015 used in vivo 2-photon microscopy and optogenetic stimulation on NG2creER transgenic mice and injected with tamoxifen which was used to separate smooth muscle cells and pericytes to avoid artifacts from non-mural cells (6). Alarcon-Martinez et. al. 2018 also claims that the tamoxifen injection could have impacted the detection of α-SMA (5). In their study, they investigated whether faster fixation techniques and/or if stabilizing filamentous actin in vivo would improve the detection of α-SMA.

To stabilize α-SMA in vivo, they used phalloidin and jasplakinolide, which are compounds that prevent filamentous actin depolymerization, on retinal capillaries prior to termination for immunostaining (5). They found that phalloidin significantly increased the detection of α-SMA in retinal capillary pericytes compared to traditional paraformaldehyde fixation performed post-termination. They also tested using methanol at -20^{o}C for snap freeze fixation which led to similar detection levels of α-SMA as phalloidin. Additionally, the use of jasplakinolide significantly increased detection of α-SMA at higher order capillaries, suggesting that α-SMA in downstream capillaries is less stable due to its lower expression compared to first order capillaries and arterioles and more prone to depolymerization. The expression of α-SMA in downstream capillary

pericytes is further confirmed again in Gonzales et. al. 2020 as immunofluorescent staining intensity in mice retina gradually decreased when reaching downstream capillaries but was still detectable. However, while they used paraformaldehyde for fixation, they did not inject mice with tamoxifen.

Finally, in line with Hill et. al. 2015, the study Hartmann et. al. 2021 also saw significant expression of α-SMA in the precapillary arteriole but was undetectable in capillaries (8). However, as mentioned previously, Hartmann et. al. 2021 lamented that ensheathing pericytes surround the precapillary arteriole rather than arteriolar smooth muscle. This is quite clear from their fluorescent staining of ensheathing pericytes and α-SMA that the morphology matches up with the traditional "bump-on-a-log" appearance often used to describe pericytes. Additionally, fluorescent images from Hill et. al. 2015 of precapillary arteriole expression of α-SMA also quite clearly have a similar "bump-on-a-log" morphology to pericytes despite indicating that these are smooth muscle cells (6).

Pericytes Induce Changes in Capillary Diameter and Red Blood Cell Flux and Loss Leads to Neurovascular Uncoupling

While there seems to be clear evidence that pericytes express α-SMA, it is also important to prove that they do in fact modulate capillary diameter. The study Hall et. al. 2014 aimed to illustrate that pericytes dilate capillaries during neuronal activity making them important for neurovascular coupling (4). In the study they identified pericytes by using NG2 DsRed transgenic mice and applied noradrenaline to cerebellar brain slices which induced a consistent constriction of pericytes. They were also able to induce pericyte dilation by using glutamate to mimic neuronal signalling. While these results are interesting, it was unclear which cells were being acted on. Therefore, they aimed to determine specific molecular mediators involved in generating pericyte tone. The eicosanoid metabolite, 20-Hydroxyeicosatetraenoic acid (20-HETE), is important for vasoconstriction and its synthesis was blocked, but it did not result in any changes in pericyte constriction caused by noradrenaline. However, blocking nitric oxide (NO) synthesis, which is an important vasodilator, reduced glutamate induced dilation. Finally, dilation remained robust when blocking 20-HETE and NO which means other mediators are at play. Blocking prostaglandin E2 receptors reduced capillary dilation.

To further validate their findings that pericytes regulate cerebral blood flow by stimulation from neurovascular mediators, Hall et. al. 2014 performed an in vivo study by electrically stimulating the whisker pad and using 2-photon imaging in the NG2 DsRed transgenic mice (4). Interestingly, they found that 1st order capillaries consistently dilated before penetrating arterioles which indicates that capillary dilation is not a response to pressure increases from the arterioles. Additionally, they compared diameter changes in capillaries in pericyte covered regions vs. no pericyte covered regions and found that when capillaries dilated, their diameter was larger in pericyte regions. A later study by Kisler et. al. 2017 had similar findings that capillary dilation occurred prior to arteriole dilation when applying electrical hindlimb stimulus also with NG2 DsRed mice (9). They also confirmed that changes in diameter were much greater in pericyte covered capillary regions compared with non-covered region.

Kisler et. al. 2017 went a step further in providing evidence of pericyte importance in cerebral blood flow which was to demonstrate changes in cerebral blood flow and neurovascular coupling with pericyte deficient mice (9). They crossed PDGFRβ$^{+/-}$ mice with NG2 DsRed mice to reduce pericyte numbers and visualize pericytes. In comparison to the controls which had similar results

as Hall et. al. 2014, the pericyte deficient mice had barely detectable and delayed dilation in response to hindlimb stimulation. They also confirmed that the neurovascular uncoupling they observed was not due to arteriole or astrocyte dysfunction but there were no differences in cerebral blood flow at the arteriole level, nor were there signs of morphological abnormalities in astrocytes. In addition to impaired or delayed dilatory reaction to stimuli, Kisler et. al. 2017 also observed that pericyte loss may result in chronic hypoperfusion or hypoxia (9). They evaluated the state of tissue oxygenation with 2-photon laser scanning microscopy of pO_2 and saw a shift away from physiological range which is typically between 20 – 40 mmHg. In the pericyte deficient mice, a large area had an pO_2 of 15 mmHg or less and in some regions at greater distance from arterioles frequently had less than 5 mmHg. They strengthened their results by showing pericyte deficient mice overall have insufficient oxygen consumption because they had higher levels of NADH than NAD+ and also had 47% higher levels of lactate in the cortex compared to control animals (9).

In contrast to Hall et. al. 2014 and Kisler et. al. 2017, the study Hartmann et. al. 2021 examined pericyte contraction and dilation of capillaries rather than only dilation and if they contribute to flow resistance (8). In the study they used optogenetic stimulation of channel rhodopsin 2 (ChR2) by crossing PDGFRβ Cre mice with ChR2-YFP mice. They found that stimulation of ChR2 expressing pericytes led to an overall decrease in capillary diameter. The constriction was enough to decrease the red blood cell velocity and which also indicates that the level of constriction induced by pericytes was enough to alter cerebral blood flow (8). The study also wanted to determine if pericyte contraction relied on contractile machinery and therefore used the Rho-kinase inhibitor, Fasudil, which leads to vasodilation of smooth muscle cells. This resulted in capillary dilation as well as an increase in red blood cell flux which indicated that capillary pericytes do utilize contractile machinery, which could potentially be α-SMA. Consistent with Hall et. al. 2014 and Kisler et. al. 2017, Hartmann et. al. 2021 also saw a significantly higher dilation rate in the precapillary arteriole which the other studies considered as a 1^{st} order capillary.

Finally, both Gonzales et. al. 2020 and Hartmann et. al. 2021 both highlighted the heterogeneity of capillary diameter and flow rate (7, 8). In Hartmann et. al. 2021 they performed optical ablation of pericytes and observed continuous capillary dilation in ablated areas along with a 2-fold increase in red blood cell flux. This result indicates that pericytes sustain blood flow resistance all over the capillary network. They also observed that capillaries that had smaller diameters prior to pericyte ablation had greater increases in dilation compared to capillaries with larger diameters which suggests pericytes can apply different levels of tone making capillary blood flow heterogenous. In Gonzales et. al. 2020 they hypothesized that applying a K^+ stimulus to induce retrograde hyperpolarizing signals to pericytes via gap junctions with the underlying endothelium would lead to increased capillary diameter (7). They found that retrograde K^+ signals modified pericyte contractility and resulted in asymmetric reactions to capillary diameter of different branches in pressurized retina samples. They also delivered K^+ in vivo to capillary junctions in NG2 DsRed transgenic mice and similarly to Hartmann et. al. 2021 they saw increases in red blood cell flux as well as asymmetric shifts in this flux which further confirms capillary flow heterogeneity (7).

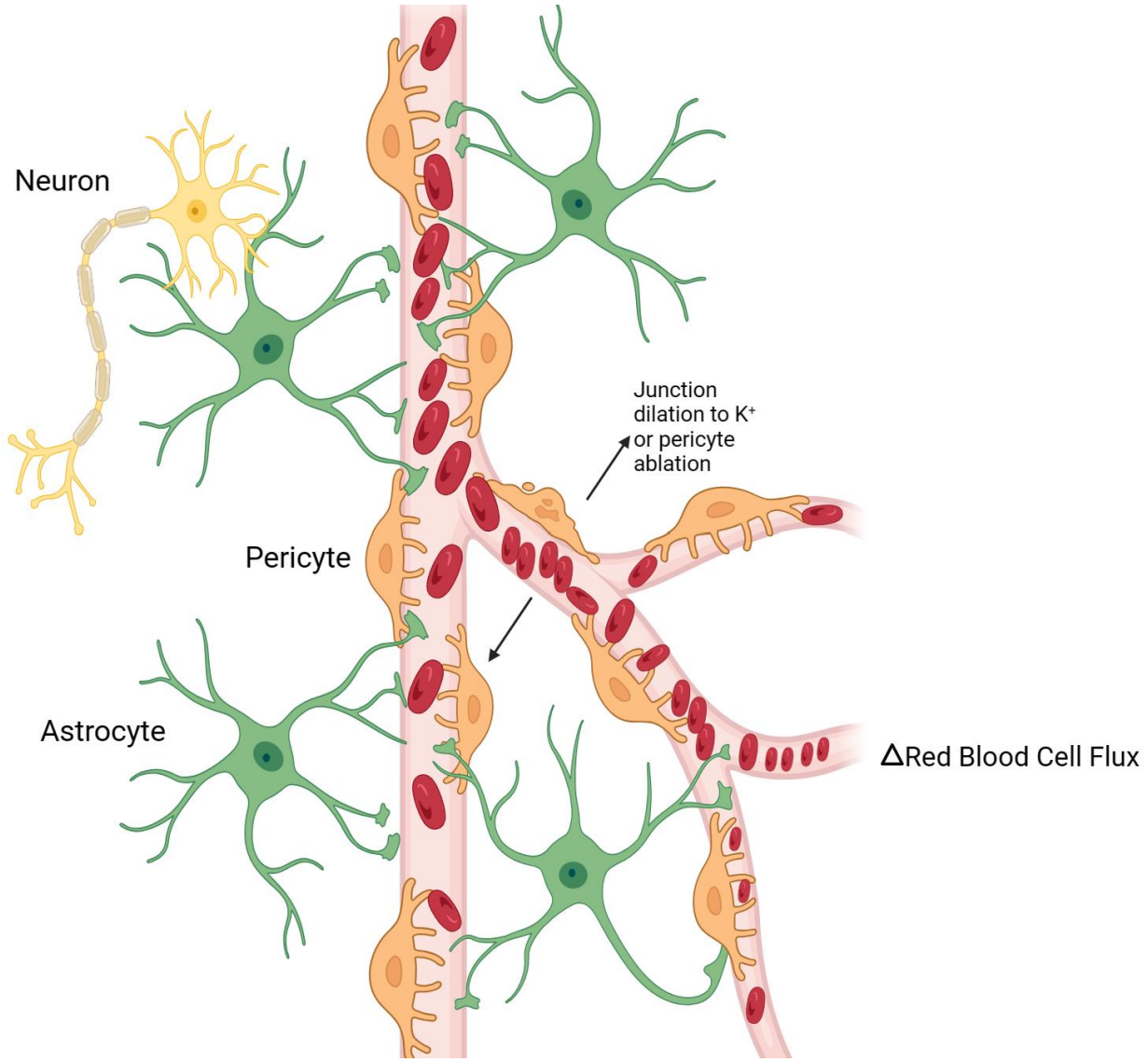

Figure 2: Changes in red blood cell flux as described in Gonzales et. al. 2020 and Hartmann et. al. 2021 (7, 8). Both studies showed that either hyperpolarization with K^+ or ablation of pericytes led to increased red blood cell flux and that capillary blood flow was heterogenous or asymmetric. This is highlighted in the image above showing that an increase in flux shifts to the first capillary junction but the number of blood cells at each branch order differs. This figure was created using Biorender.com

Pericytes Regulate Cerebral Blood Flow via K_{ATP}-Channel and TMEM16A Ca^{2+} Channel Activation

Since much of the focus of pericyte function of cerebral blood flow has been on pericyte contractility and ensheathing pericytes, what about pericytes in higher order capillaries? How can they regulate cerebral blood flow with minimal to no detectedN expression of α-SMA as shown in previous sections? The capillary dilation from hyperpolarizing K^+ signals to pericytes presented in Gonzales et. al. 2020 is further elucidated in Hariharan et. al. 2022 where they look specifically at K_{ATP} channels expressed on thin strand pericytes (7, 10). As described previously, thin strand pericytes have long but few projections and are typically expressed further away from capillary-arteriole junctions where ensheathing pericytes are usually expressed. In Hariharan et. al. 2022 they aimed to investigate how thin strand pericytes metabolically regulate cerebral blood flow (10). In the study they applied the selective K_{ATP} inhibitor, pinacidil, thin strand pericytes in NG2 DsRed

transgenic mice which led to dilation of upstream penetrating arterioles and an increase in blood flow to capillaries. They concluded that the connection between pericytes and endothelial cells with gap junctions must be how the hyperpolarizing signal is communicated back to the penetrating arterioles to dilate (10). To prove this, they applied pinacidil to penetrating arterioles and capillary sections without a pericyte and saw no effect. They also bred mice with a nonfunctional subunit to remove K_{ATP} channel conductance in pericytes and again saw that pinacidil did not dilate penetrating arterioles or increase capillary blood flow.

In addition to these results, Hariharan et. al. 2022 explored potential mechanisms that may lead to pericyte hyperpolarization via K_{ATP} channels (10). They hypothesized that pericytes may depolarize in response to shifts in energy substrates and glucose. Therefore, they removed capillaries from NG2 DsRed mice to measure the differences in membrane potential of pericytes when extracellular glucose concentrations were shifted. They observed that pericyte membrane potential was relatively stable between glucose concentrations of 1 and 4 mM, but that they were very hyperpolarized with 0 – 0.75 mM glucose. Additionally, they blocked glucose transporter 1 (GLUT1) which led to significant hyperpolarization as seen with glucose concentrations below 1 mM which led to significant penetrating arteriole dilation. Overall, their results indicate that pericytes may also regulate cerebral blood flow by detecting changes in the surrounding environment to signal for upstream arteriole dilation, however, additional studies are needed for confirmation.

Another channel which may be implicated in capillary pericyte regulation of cerebral blood flow is the Ca^{2+}-gated Cl^- channel called TMEM16A which is investigated in Korte et. al. 2022 (11). In the study they aimed to examine TMEM16A as an augmenter of pericyte contractility triggered by Ca^{2+}. They used endothelin-1 and the thromboxane analogue U46619 to activate Gq protein coupled receptors (GPCR) on pericytes to constrict capillaries. This led to increases in intracellular Ca^{2+}. They then applied MONNA and Ani9 which are inhibitors of the TMEM16A channel which significantly reduced capillary constriction induced by endothelin-1 and U46619. They also found that the application of Ani9 reduced the rise in intracellular Ca^{2+} suggesting that there is a relationship between activation of TMEM16A and amplification of rising intracellular Ca^{2+} concentration in pericytes likely through Ca_v channels. Finally, because Korte et. al. 2022 considered that TMEM16A plays a role in depolarizing mechanisms in pericytes during GPCR activation, they tested the effects of altering the Cl^- gradient on capillary diameter (11). They used bumetanide which is an inhibitor of the NKCC1 Cl^- importer to reduce any depolarization influenced by TMEM16A and then subjected pericytes to endothelin-1 treatment. This resulted in a 4-fold reduction in capillary constriction. Overall, the study concluded that pericyte contraction is very reliant on the Cl^- transmembrane gradient.

4.3 Pericytes Constrict Capillaries During and After Cerebral Ischemia Contributing to the "No Reflow" Phenomenon

So far, this review has covered several studies that have provided evidence that pericytes play a role in regulating cerebral blood flow in healthy animals. However, a few of the previously presented studies have also covered how pericytes can also impede cerebral blood flow, specifically following an ischemic stroke and how they might be the cause of the "no reflow" phenomenon. The study Hall et. al. 2014 they investigated the effects of ischemia on pericytes in cortical brain slices (4). With live imaging they found that capillaries constricted within 15 minutes of exposure in restricted regions. Pericytes that were outside of exposed regions were stable for at least one hour. Additionally, they blocked AMPA/kainite and NMDA receptors which reduced

pericyte death by half which indicates that excitotoxicity may promote pericyte death. They also performed oxygen-glucose deprivation so that ATP production could return when oxygen and glucose were restored. They found that during deprivation approximately 40% of pericytes died in one hour, but during one hour of reperfusion pericyte death increased 1.5-fold but blocking ionotropic glutamate receptors and removing extracellular Ca^{2+} significantly reduced pericyte death (4). These results overall support the idea that pericytes may continue to impede cerebral blood flow even after it has been reestablished following ischemic insult and that reducing excitotoxicity reduces pericyte death and could improve recovery.

The study Korte et. al. 2022 elucidates this further by offering possible mechanisms causing pericyte death as well as suggesting that TMEM16A could be a potential therapeutic target to reduce pericyte death in stroke (11). As described previously, the TMEM16A inhibitor, Ani9, greatly reduced pericyte contraction and therefore, they wanted to investigate if Ani9 could reduce pericyte death during and following oxygen glucose deprivation in cortical brain slices. They found that Ani9 caused significant delays in pericyte induced capillary constriction and reduced pericyte death from 50.1% to 28.7% (11). They explained that this was likely due to reduced intracellular Ca^{2+} levels that would normally be unable to be pumped out during ischemia due to lack of ATP. Inhibiting TMEM16A would help to delay Ca^{2+} accumulation since they would be unable to promote the activation of Ca_v channels. They also applied Ani9 after common carotid artery occlusion (CCAO) in vivo and saw similar results of less Ca^{2+} accumulation in pericytes (11). Additionally, they observed improved cerebral blood flow immediately after CCAO and 70-90 minutes reperfusion. Finally, they observed that blocking TMEM16A with Ani9 following CCAO resulted in an overall reduction in ischemic infarct size, hypoxia, and neuronal injury even beyond the acute phase (11). These findings suggest that reducing pericyte death is of therapeutic benefit for improving ischemic stroke outcomes and cerebral blood flow.

4.4 Conclusion

In conclusion, while the function of pericytes in cerebral blood flow has been a controversial subject, the majority of most recent findings in the literature of the last 10 years seem to support the idea. However, this does not mean there are not issues to resolve, the most significant being the importance of being able to distinguish between pericytes and arteriolar smooth muscle. Additionally, coming to an agreement on whether the term precapillary arteriole or first order capillary is most appropriate would aid significantly in avoiding confusion. Despite these issues with morphological characterization and terminology, the evidence of pericyte contribution to cerebral blood flow is robust, specifically in ensheathing pericytes which express α-SMA.

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