CAPILLARY PERICYTES

A tense relationship between capillaries and pericytes

Most of the cerebral microcirculation is comprised of capillaries that are lined with pericytes, but the influence of pericytes on local blood flow was not previously established. A new study by Hartmann and colleagues uses selective optical ablation or activation to demonstrate that capillary pericytes exert both static and slow types of regulation on capillary diameter to affect flow, which are distinct from canonical rapid regulation by arteriole smooth muscle.

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An evolutionary success of the mammalian brain is its higher metabolic capacity compared to other types of animals. The neocortex of mammals relies upon small, deformable erythrocytes, tightly transiting an extremely dense capillary network. This enables the fueling of intense neuronal processing with more oxygen and glucose than is possible in lower phylogenetic organisms, such as reptiles and birds, which have larger nucleated red blood cells (RBCs)[1]. However, a susceptibility of the mammalian brain is that metabolic supply–demand mismatch has severe functional consequences, as seen during stroke, seizure, spreading depression, and several types of dementia.

Within mammals, the volume of oxygenated blood that can reach brain cells per unit time is mostly determined by the flow resistance of the supplying vasculature. It is estimated that the majority of resistance originates from capillaries[2], but to what extent capillaries are capable of actively controlling flow resistance by changing diameter is a long-standing debate. Canonically, vascular smooth muscle cells (VSMC) in arterioles can rapidly constrict or dilate the vessel lumen to regulate cerebral blood flow (CBF). In contrast, capillaries, comprised of endothelial cells wrapped by pericytes, are at the center of scientific 'turbulence' over their exact contribution to CBF and even how pericytes are defined[3].

It has been repeatedly demonstrated that the initial, transitional segments of 'small vessels' arising from penetrating arterioles in the neocortex can change diameter by contractile VSMC–pericyte hybrid cells called ensheathing pericytes[4,5]. In contrast, the greater population of 'thin-strand' pericytes found in more distal capillary segments (more than four branch orders off the penetrator) and covering 96% of all microvasculature, were claimed to be passive[6] or not actively contributing to physiological cerebral perfusion[7]. The new study by Hartmann et al.[8] in *Nature Neuroscience* advances our view by demonstrating that distal capillary pericytes

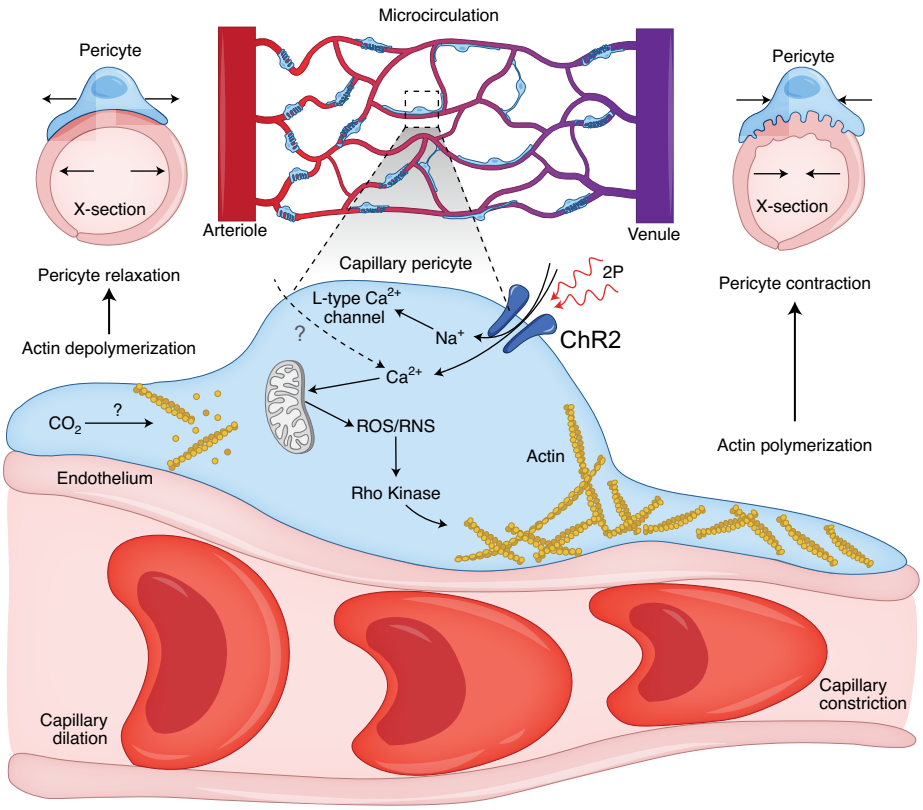

Fig. 1 | Thin-strand pericytes provide slow regulation of capillary tone. Capillary pericytes with a thin-strand morphology are located on high-order capillaries (top center). These cells contain low amounts of α-SMA, but enough to generate tension on the capillary wall when polymerized and to set resting capillary diameter (bottom). Optogenetic stimulation (2P) elevates intracellular Ca^{2+} and free radical production, which together activate Rho-kinase to promote actin filament assembly and actomyosin coupling. The force generated cinches the endothelium and narrows the capillary lumen (right). Actin depolymerization reduces pericyte tension and dilates the underlying capillary, as seen during mild hypercapnia (left). X-section, cross-section; ROS, reactive oxygen species; RNS, reactive nitrogen species.

provide vascular tone and slowly modulate flow resistance in vivo. This finding will catalyze future work on the physiological functions of brain capillary beds, as well as the translational potential of pericytes in CBF-associated diseases and conditions.

Cleared mouse cortical tissue was used for immunostaining of α-smooth muscle actin (SMA), an essential component of contractility. Staining confirmed that α-SMA is undetectable in distal capillary pericytes of the mouse cortex. Undeterred, the authors explored what happens to capillary diameter when individual channelrhodopsin-2 (ChR2)-expressing pericytes (under the platelet-derived growth factor receptor beta (PDGFR-β) promoter) are selectively light-activated for 1 min through a closed cranial window over the sensory cortex of mice. They optically stimulated one pericyte at a time to avoid activating proximal contractile cells, which can indirectly change downstream blood flow in the investigated capillaries. In contrast to previous attempts, where ChR2-activation of pericytes for less than 20 s had no effect on capillary diameter[6], they observed slow, progressive vessel contraction (~20%) adjacent to both the pericyte soma and far-extending processes along the capillary, with a subsequent decrease in RBC velocity and flux (blood cells per second). This constriction had much slower (2.5×) kinetics than those recorded by optogenetic activation of upstream ensheathing pericytes (Fig. 1). Additional work to exclude the possibility of artifacts from pericyte swelling and phototoxicity revealed that capillary constriction occurred irrespective of the chosen anesthetic, developed similarly at various cortical depth by matching laser powers, was not accompanied by upstream precapillary constriction, preceded the drop of RBC flux, and was negligible in transgenic control mice. RBC stalling was also observed in a third of capillaries constricted by ChR2 activation.

To identify the molecular basis of the observed phenomena, the authors found a dose-dependent abolishment of optogenetic-induced pericyte constriction with the Rho-kinase inhibitor fasudil. They assert that the effect of fasudil on pericytes was likely not due to classical inhibition of actomyosin coupling, but occurred by preventing cytoskeletal actin polymerization, a process recently shown to occur in retinal pericytes that contain actin monomers but show low α-SMA expression with standard immunostaining[9]. Reactive oxygen species appear to link ChR2-induced pericyte depolarization and Rho-kinase activation, by preventing capillary constriction with the antioxidant N-acetylcystein (Fig. 1). An important aspect of the constrictive pericyte response is reversibility. Indeed, capillary diameter recovered to baseline over minutes after optogenetic activation, contrary to the rapid recovery (seconds) of upstream vascular segments. This indicates an active, albeit slow, bidirectional control of capillary diameter by pericytes.

An important question is whether these slow pericyte responses exist during physiologically relevant challenges. Carbon dioxide, the byproduct of aerobic metabolism, is well known to increase CBF when elevated in the blood or in brain parenchyma. Hartmann et al. found that the inhalation of 5% CO_2 for 6 min elicited a transient dilation of precapillary arterioles and more gradual dilation in capillaries, confirming the observations of recent studies[10,11]. Likewise, capillaries constricted back to baseline more slowly than upstream arterioles after the CO_2 challenge.

Finally, Hartmann and colleagues aimed to explore the physiological role of capillary pericytes at rest by examining the consequences of pericyte loss. They monitored capillary diameter 3 days after single pericytes were selectively killed using a high-energy laser beam focused on their cell bodies. This two-photon optical ablation resulted in an isolated enlargement of the capillary lumen and doubled RBC flux at sites that were previously wrapped by pericytes. Focusing the laser adjacent to pericytes had no such effect. As most pericyte somata are integral parts of the capillary wall, phototoxicity might have extended to immediately adjacent endothelial cells and astrocyte endfeet. To address this potential caveat, they targeted 'bridging' and 'floating' pericytes (cell bodies located in the capillary wall or in the parenchyma, respectively) that contacted remote capillary segments with far-reaching processes[12] and were able to reproduce the same phenomena: capillary regions covered by the irradiated pericyte's processes were distended. Three weeks after ablation, capillaries regained tone as neighboring pericyte processes invaded the denuded segments. Interestingly, the thinner a capillary was at rest, the more it dilated after ablation, confirming that resting capillary tone and flow resistance is set by pericytes to various degrees, underlying capillary blood flow heterogeneity.

Although the study resolves and integrates several puzzling questions about pericyte contractility, important contradictions need to be addressed in future studies to understand which functional, metabolic, hormonal, and pathological signals stimulate capillary tone changes by pericytes. One pressing question is whether low α-SMA-containing capillary pericytes contribute to functional hyperemia by reducing capillary tone when neuronal activity increases. Both VSMCs and ensheathing pericytes rapidly relax when local neurons are activated[4,5], but active dilation of distal capillaries is controversial, as one study reported no diameter response to sustained, 30 s sensory stimulation[6], while a more recent publication found rapid dilations and constrictions to brief retinal stimulation (although it is unclear what type of pericyte was studied)[12]. If not functional hyperemia, perhaps capillary pericytes help redistribute tissue O_2 regionally to groups of neurons experiencing higher or lower metabolic demands on slower timescales, such as during plastic changes. Alternatively, actin polymerization in retinal pericytes was evoked by norepinephrine injection[9]; thus, interrogating the contribution of ascending noradrenergic fibers from the locus coeruleus to pericyte tone control could be a promising approach. Understanding how other cell types in the vicinity of pericytes—RBCs, endothelial cells, astrocytes, and microglia—could regulate their tone will also be informative. Finally, the transient dilation of capillary pericytes to a relatively mild CO_2 challenge suggests that pericytes respond to metabolic signals. Contradicting this, a spreading wave of cortical depolarization, which is accompanied by a massive release of glutamate, K^+, metabolites, and vasoactive substances leading to large arteriole diameter changes, did not elicit tone changes in capillary pericytes[6].

In providing convincing evidence of the regulatory effects of pericytes on capillary tone, these findings highlight new directions and potential targets for CBF-associated brain diseases. In the context of stroke, ischemia-reperfusion constricts and can kill ensheathing pericytes[4,13], but it is disputed whether distal capillaries constrict[12,14] or not[6], and what the impact that may have on ischemia. Capillary constriction was also shown to be a leading factor for Alzheimer's disease progression[15]. New studies are warranted to clarify whether α-SMA-negative thin-strand pericytes constrict capillaries differently from α-SMA-positive pericytes by endothelin-1 and reactive oxygen species in response to soluble amyloid-β accumulation. Finally, slow-constricting pericytes could respond to inflammatory mediators released from glia, entrapped neutrophils, or from the endothelium during infections or diabetes. The field is still ripe for exploration, though it must proceed under a guiding principle of carefully defining pericytes by morphology, expression profile, and location. This

study has convincingly demonstrated that thin-strand pericytes have a 'tense relationship' with capillaries, squeezing out new questions and relaxing the barriers for new debate. ❐

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Published online: 21 April 2021
https://doi.org/10.1038/s41593-021-00853-1

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Competing interests

The authors declare no competing interests.