Calcium Signalling in Pericytes

Theodor Burdyga  Lyudmyla Borysova

Department of Cellular and Molecular Physiology, Institute of Translational Medicine, University of Liverpool, Liverpool, UK

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Abstract
Recent advances in pericyte research have contributed to our understanding of the physiology and pathophysiology of microvessels. The microvasculature consists of arteriolar and venular networks located upstream and downstream of the capillaries. Arterioles are surrounded by a monolayer of spindle-shaped myocytes, while terminal branches of precapillary arterioles, capillaries and all sections of postcapillary venules are encircled by a monolayer of morphologically diverse pericytes. There are physiological differences in the response of pericytes and myocytes to vasoactive molecules, suggesting that these two vascular cell types could have different functional roles in the regulation of local blood flow. The contractile activity of pericytes and myocytes is controlled by changes of cytosolic free Ca\(^{2+}\) concentration. In this short review, we summarize our results and those of other authors on the contractility of pericytes and their Ca\(^{2+}\) signalling. We describe results regarding sources of Ca\(^{2+}\) and mechanisms of Ca\(^{2+}\) release and Ca\(^{2+}\) entry in control of the spatiotemporal characteristics of the Ca\(^{2+}\) signals in pericytes.

Introduction

The microcirculation plays a central role in the control of tissue blood supply. Precapillary and postcapillary microvessels control the direction and rate of blood flow, the capillary filtration and the exudation of blood components into the tissues, i.e. transmigration [1]. The balance of the vascular tone of the precapillary arterioles and postcapillary venules is a crucial determinant of capillary hemodynamics. The mechanisms of vasomotor activity in response to central and local factors, especially in the ‘pericytic’ part of the microcirculation, are largely unknown. The pericyte-containing part of microcirculation includes the distal parts of the precapillary arterioles, the capillaries and all sections of the postcapillary venules [2–4]. Much remains to be learned about how the microvasculature is functionally organized. The most investigated functional property of pericytes has been their ability to contract. Ca\(^{2+}\) signalling controls the contractile apparatus of pericytes. The advent of Ca\(^{2+}\)-sensitive fluorescent dyes, together with video-based imaging and photometric techniques opened the door for the investigation of pericyte Ca\(^{2+}\) signalling. The mechanisms of Ca\(^{2+}\) entry and Ca\(^{2+}\) release, which shape the spatiotemporal profiles of agonist-induced Ca\(^{2+}\) signals in pericytes, are under current investigation. Ca\(^{2+}\) signalling in cultured peri-
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cytes or in isolated microvessels is measured primarily with a photometric (‘blind’) system using the ratio-metric Ca$^{2+}$-sensitive indicators Indo-1 [5, 6] or Fura-2 [7–11]. Confocal Ca$^{2+}$ imaging has brought many advantages to the study of Ca$^{2+}$ signalling in situ. Confocal images of vessels permit the visualization of individual vascular smooth muscle cells, pericytes and endothelial cells, and so quantification of changes of Ca$^{2+}$ signals in these cells can be made and then correlated with mechanical or electrical events [12–18]. In this review, we attempt to summarize our results and those of other authors over the last 2 decades in the field of Ca$^{2+}$ signalling in pericytes.

**Topology, Morphology and Contractility of Pericytes**

A lengthy history of morphological studies on pericytes has led to a firm conclusion that they represent a distinctive cell population of the microvasculature of the brain [19], retina [2, 20, 21] and kidney [22], with ubiquitous distribution and morphology [2, 23]. However, morphological, functional and molecular expression studies on pericytes in the peripheral tissues are generally lacking. Traditionally, pericytes are defined as extensively branched cells. Based on location and histological characteristics, three different types have been identified: precapillary (arteriolar), capillary and postcapillary (venular) [2]. This topology and morphology has been confirmed by scanning electron microscopy studies of microvessels in different tissues [3, 4, 23–26] and recently by live confocal imaging of ureteric microvascular network in situ (fig. 1) [15].

**Precapillary Pericytes**

Precapillary pericytes are located in the distal part of the precapillary arterioles or initial segments of arteriolar capillaries. Morphologically, they have thick cell bodies giving several finger-like processes tightly wrapping around the endothelium (fig. 1Ai, Ci). There is general agreement that precapillary pericytes are contractile cells.
They express smooth muscle α-actin (α-SMA) [15, 20, 27–29], tropomyosin [30] and cGMP-dependent protein kinase [31] (fig. 1Dii). Expression of α-SMA in the pericytes of precapillary arterioles has recently been confirmed in vivo in transgenic albino mice [29]. Precapillary pericytes from human placenta and mouse skin also stain positive in vitro for a pericyte marker chondroitin sulphate proteoglycan (NG2) [32]. Thus the morphological appearance of the precapillary pericytes and their molecular composition are consistent with a contractile phenotype. Considering not only their unique anatomical location, but also their contact with the blood and interstitial fluid, the pericytes of precapillary arterioles represent prominent controlling elements that regulate tissue blood supply and it is suggested that they act as precapillary sphincters [15, 33, 34]. A movie showing changes in the concentration of \([Ca^{2+}]\) and contraction induced by endothelin-1 (ET-1) of precapillary pericytes in situ is available in the supplemental material provided for our recent publication [15].

**Postcapillary Pericytes**

All sections of postcapillary venules are surrounded by fibroblast-like pericytes, with stellate cell bodies giving slender cell projections. These projections appear to be randomly oriented with respect to the vessel axis, and overlap or attach to each other, forming a complicated and dense meshwork (fig. 1Aiv, Ciii–v). They stain positive for α-SMA [15, 17, 20, 28, 29, 35] (fig. 1Div) but negative for NG2 and thus are classified as an NG2-α-SMA phenotype [32, 35]. Information has been accumulated providing evidence that postcapillary pericytes are contractile cells and can perform several functions in the microcirculation including the regulation of blood flow and perfusion [15, 17, 36], the opening of interendothelial junctions [37] and junction protection from overstretching (‘umbrella-like’ disposition over interendothelial junctions) [38]. Movies showing contractile activity in venular pericytes are available in the supplemental material provided for several recent publications [15, 17].

**Capillary Pericytes**

The capillary pericytes of all tissues have elongated cell bodies with two long slender processes on each side running parallel to the long axis of the capillary tube that tapers to smaller processes which partly encircle the capillary wall [2], providing the appearance of pericytes cradling the capillary tubes (fig. 1Aiii, Cii). There are conflicting data as to whether capillary pericytes are indeed contractile cells. They stain positively for NG2 but in most cases negatively for α-SMA [15, 20, 28, 35, 39] (fig. 1Diii). α-SMA is not expressed in the capillaries of transgenic albino mice [29]. The presence of contractile proteins in capillary pericytes is quite controversial and seems to depend upon the vascular bed and their position within the capillary network. For example, smooth muscle myosin is found in capillary pericytes from the heart, diaphragm, pancreas and intestinal mucosa [30] but not in the brain and retina [40]. The contractile activity of the pericytes of midcapillaries is heterogeneous and requires further investigation [21]. Online movies of retinal capillary pericyte contraction induced by a variety of extracellular signalling molecules are available in the supplemental material provided for several recent publications [8, 9, 41, 42].

**Pericytes Are Electrically Excitable Cells**

Electrophysiological and pharmacological studies have revealed that pericytes are electrically excitable cells [10, 43–45]. The resting membrane potential of pericytes ranges from –32 to –70 mV [43, 45] and can be modulated by different ion channels present in their plasma membrane such as Ca\(^{2+}\)-activated Cl\(^–\) channels (Cl\(_{Ca}\)) [18, 46], Ca\(^{2+}\)-activated K\(^+\) channels (K\(_{Ca}\)) [45], ATP-sensitive K\(^+\) channels (K\(_{ATP}\)) [47], inwardly rectifying K\(^+\) channels (K\(_{IR}\)) [43, 45], voltage-dependent K\(^+\) channels (K\(_{V}\)) [45], voltage-gated Na\(^+\) channels [48], nonselective cation channels [21] and transient receptor potential channels [49]. Voltage-clamp experiments performed on pericytes in culture [10] or on isolated microvessels [10, 44, 45] show that pericytes contain L-type voltage-gated Ca\(^{2+}\) channels (VGCC). However, comparative studies performed on the freshly isolated retinal microvascular complexes [44] and kidney tissue slices [45] show that VGCC-mediated changes in ionic currents are markedly (i.e. 7.5 times) greater in myocytes than in pericytes [44, 45]. These findings indicate that potential-dependent, dihydropyridine-sensitive VGCCs play a significant role in excitation-contraction coupling in arteriolar myocytes but may either be poorly distributed or physiologically silent in pericytes. It is not known whether molecular isoforms of VGCC are expressed in pericytes. In rat retinal pericytes, this difference in VGCC function is, in large part, due to the selective inhibition of the VGCCs in the pericytes by endogenous spermine, a polyanine known to inhibit L-type calcium channels [44].
Membrane potential plays a central role in the control of [Ca\(^{2+}\)]\(_{i}\), concentration by modulating the influx of Ca\(^{2+}\) via L-type voltage-dependent Ca\(^{2+}\) channels in smooth muscle cells and pericytes. K\(^{+}\) channel blockers TEA or BaCl\(_{2}\) induce the depolarization of pericytes and the constriction of the vasa recta, although the types of K\(^{+}\) channels present in vasa recta pericytes have not been discussed [45]. In cultured bovine retinal pericytes, repetitive spike-like action potentials are induced by BaCl\(_{2}\) and norepinephrine. These action potentials depend on the presence of extracellular Ca\(^{2+}\) and are inhibited by the Ca\(^{2+}\) antagonist nifedipine [43]. High-K\(^{+}\) induces depolarization of the cell membrane and activates Ca\(^{2+}\) entry via nifedipine-sensitive VGCC in the pericytes of precapillary arterioles, the capillaries and postcapillary venules of rat ureteric microvascular beds in situ [15], the capillaries of rat retina [44] and the kidney vasa recta [45]. Comparative studies performed on the freshly isolated retinal microvascular complexes [44], kidney tissue slices [45] and ureteric microvascular networks in situ [15] show that VGCC-mediated changes in [Ca\(^{2+}\)]\(_{i}\), signals and contractility induced by high-K\(^{+}\) depolarization are 4–6 times smaller in pericytes than in myocytes. In pericytes, Ca\(^{2+}\) transients and contractile responses induced by agonists are 4–5 times larger than those induced by high-K\(^{+}\) depolarization [15, 45]. Collectively, the existing data indicate that pericytes express functional L-type VGCC, but their molecular entity is not known. The role of VGCC in excitation-contraction coupling in pericytes is much smaller than in myocytes, suggesting that there is heterogeneity in the functional distribution of VGCCs between myocytes and pericytes.

**VGCC-Dependent Intercellular Ca\(^{2+}\) Signalling**

The longitudinal conduction of vasomotor responses coordinates changes in blood vessel diameter and blood flow distribution in complex microvascular networks [50, 51]. Vasconstrictor or vasodilator motor signals can spread through gap junctions connecting media and endothelial cells in both directions along the vessel length which coordinates the blood flow in the proximal and distal vascular segments in the microcirculation [50–57]. The monolayer of endothelial cells surrounded by a single layer of myocytes and pericytes establishes a perfect structural precedent for longitudinal signalling along the vessel axis (fig. 1B).

Elegant electrophysiological experiments performed on isolated retinal microvascular complexes [9, 58–61] demonstrate that capillary pericytes are electrically coupled to the myocytes of upstream precapillary arterioles and constitute a functional unit within which the highly dynamic cell-to-cell bidirectional transmission of electrical signals via heterocellular and homocellular gap junctions occurs.

Since both myocytes and pericytes express VGCC and are electrically coupled, the transmission of intercellular depolarization-driven Ca\(^{2+}\) signals from myocytes to pericytes via homocellular and/or heterocellular gap junctions is possible. The rapid transmission of intercellular Ca\(^{2+}\) signals from arteriolar myocytes to capillary and venular pericytes along microvessel axis, which requires depolarization, L-type Ca\(^{2+}\) channels and gap junctions, has recently been shown in ureteric microvascular networks in situ [15]. The intercellular Ca\(^{2+}\) signals between the pericytes of isolated vasa recta renis have also been recently observed [62]. However, it is not clear whether an axial spread of intercellular Ca\(^{2+}\) signals from myocytes to pericytes involves the endothelium.

Pericytes are in close contact with the interstitial fluid and can act not only as transmitters but also as possible generators of depolarizing or hyperpolarizing electrical signals. These signals, induced by different metabolites, can be transmitted to myocytes of upstream arterioles and converted into vasomotor responses. Depolarization in pericytes can be achieved by activation of Ca\(^{2+}\)-activated Cl\(^{-}\) channels via mobilization of [Ca\(^{2+}\)]\(_{i}\), by different vasoactive factors [18, 21]. The membrane depolarization induced by activation of Cl\(^{-}\)\(_{Ca}\) can reach a threshold of VGCC activation and generate additional depolarization caused by Ca\(^{2+}\) influx. Hyperpolarization in pericytes can be produced by activation of different types of K\(^{+}\) channels [63]. In contrast, endothelial cells can generate mainly hyperpolarizing signals via the activation of K\(^{+}\) channels in response to the activation of endothelial cell Ca\(^{2+}\) signalling induced by at least acetylcholine [64–70]. This hyperpolarization is transmitted to upstream arterioles via myoendothelial gap junctions, although the involvement of parallel heterocellular gap junctions between pericytes and myocytes cannot be excluded. In the retinal microvascular complex, some agonists and lactate selectively and strongly inhibit the axial transmission of electrical signals from the capillary network to the arteriolar network [60]. As a consequence, the agonist-induced depolarization generated
in capillary pericytes remains localized to the capillary network and does not spread to upstream arterioles. Thus, the functional status of gap junctions may play a key role in determining the contractile responses of pericyte- and myocyte-containing microvessels to electrical and chemical signals. One of the most intriguing findings is that the noncontractile pericytes of midcapillaries respond with the elevation of \([Ca^{2+}]\) to various vasoactive signals [15, 21]. Here, the question arises as to the possible physiological role of the \(Ca^{2+}\) signalling in noncontractile pericytes. Both contractile and noncontractile pericytes express \(Ca^{2+}\)-sensitive Cl\(^-\) channels and can thus generate depolarizing currents in response to the elevation of \([Ca^{2+}]\) [18, 46]. Due to efficient gap junction-mediated transmission between pericytes and myocytes in the microvasculature [15, 58, 60, 71], a voltage change generated in the noncontractile pericytes of the capillary network can result in voltage-driven vaso-motor responses in VGCC-rich upstream precapillary arterioles. One can hypothesize that the waves of depolarization induced by the transient activation of Cl\(_{ca}\) channels by \(Ca^{2+}\) oscillations in pericytes can act as pacemakers of conducted vasomotion in upstream arterioles. Conversely, hyperpolarizing currents caused by the activation of K\(^+\) channels (e.g. K\(_{ATP}\) channels) can be expected to produce conducted vasodilation. Overall, the existing data indicate that pericytes and myocytes are electrically coupled. Cell-to-cell transmission of \(Ca^{2+}\) signals via gap junctions is present and can be modulated by different vasoactive signals.

**\(Ca^{2+}\) Signalling Induced by GPCR Agonists**

Pericytes in the microvascular bed respond to a broad range of local and central vasoconstrictors, which act mainly via binding to G-protein-coupled receptors (GPCR) producing complex \(Ca^{2+}\) transient involving \(Ca^{2+}\) release and entry pathways [72]. Microfluorometry has been used to record average changes in the concentration of \([Ca^{2+}]\), in the myocytes of isolated retinal arterioles [73], pericytes in culture [5, 6, 10, 74] and isolated microvessels [8, 9, 45, 75]. These data demonstrate that GPCR agonists [e.g. ET-1, (Arg\(^{8}\))-vasopressin (AVP), angiotensin II (Ang II), norepinephrine and ATP] evoke stereotypic \(Ca^{2+}\) transients, consisting of an initial spike followed by a sustained plateau component. It was previously thought that in both myocytes and pericytes, contraction is initiated by sarcoplasmic reticulum (SR) \(Ca^{2+}\) release and maintained by \(Ca^{2+}\) influx [8, 73]. Confocal microscopy of intact blood vessels has shown that in many blood vessels in vitro and in situ, GPCR agonist-induced contractions are controlled mainly by asynchronous \(Ca^{2+}\) wave-like oscillations. In individual myocytes, these \([Ca^{2+}]\) waves can be observed in \(Ca^{2+}\)-free solution for a relatively long time (5–30 min) [12–15, 76]. The asynchronous wave-like \(Ca^{2+}\) oscillations are now recognized as a common mode of \(Ca^{2+}\) signalling in the myocytes of arteries and arterioles [12–15, 77]. In some blood vessels, \(Ca^{2+}\) influx is needed to maintain these \(Ca^{2+}\) oscillations [13–15].

Confocal microscopy of the vasa recta [18] and intact ureteric microvascular networks in situ [15] demonstrate that the pericytes of the kidney and ureteric microvessels respond with \(Ca^{2+}\) oscillations when stimulated by some GPCR agonists. For example, ET-1 induces \(Ca^{2+}\) oscillations in arteriolar myocytes and produces nonoscillatory, single, slowly decaying \(Ca^{2+}\) transient insensitive to the removal of external \(Ca^{2+}\) in the pericytes of the ureter [15], retina [5] and brain capillaries [6] of rats. In contrast, AVP evokes \(Ca^{2+}\) oscillations in both the myocytes and pericytes of ureteric microvessels [15]. The extent of arteriolar constriction in the rat ureter induced by ET-1 and AVP correlates with the frequencies of \(Ca^{2+}\) oscillations in myocytes and is similar for both agonists [15]. In pericyte-containing microvessels, the value of constriction depends on the type of agonist used and the \(Ca^{2+}\) signal produced. Prolonged ET-1 induced \(Ca^{2+}\) spikes produce a much stronger and longer constriction than low-frequency AVP-induced \(Ca^{2+}\) oscillations [15]. Several factors, such as inositol trisphosphate (IP\(_3\)) concentration, SERCA (SR/ endoplasmic reticulum \(Ca^{2+}\)-ATPase) pump activity, the expression and distribution of different IP\(_3\) receptor (IP\(_{3}\)R) isoforms as well as the sensitivity of the contractile machinery to \(Ca^{2+}\), may account for these differences, but this needs further investigation. In summary, the data obtained indicate that pericytes are more functionally heterogeneous than myocytes with respect to agonist-induced \(Ca^{2+}\) signalling and contractility, which suggests that they play different functional roles in the regulation of local blood flow.

**\(Ca^{2+}\) Release from the SR**

Accumulated data indicate that GPCR agonists such as ET-1, AVP and Ang II, upon binding to their receptors on the plasma membrane of pericytes, activate phosphoinositide phospholipase C-\(\beta\) which then hydrolyses phosphatidylinositol-4,5-bisphosphate to yield IP\(_3\) [5, 14, 15, 17]. In turn, IP\(_3\) initiates \(Ca^{2+}\) release from the endo-
plasmic reticulum through binding to IP3Rs, the molecular identity of which has not yet been identified in pericytes [72]. The agonist-induced Ca2+ signal in pericytes appears as a Ca2+ wave which is initiated in several processes and propagates to the cell body [15]. The second family of Ca2+ release channels on the SR encompasses the ryanodine receptor channels (RyRs). Though RyRs have been identified in the myocytes of large arteries and arterioles, there is a distinct lack of evidence for the expression of functional RyRs in pericytes. Both caffeine, an activator of RyRs, and ryanodine, a selective inhibitor of RyRs, have no effect on agonist-induced Ca2+ signals in all types of pericytes [15]. Our recent work [15] supports the conclusion that RyRs do not play a major role in mobilizing Ca2+ from the SR in pericytes. However, it is not yet known whether the pericytes of other tissues express functional RyRs.

**Ca2+ Entry**

Voltage-independent mechanisms of Ca2+ entry induced by different agonists have been reported for pericytes of the retina [21] and the vasa recta of the kidney [45, 75, 78]. It has been suggested that intracellular nonselective cation channels play a role in the control of Ca2+ influx from an extracellular space in retinal pericytes [46]. In the pericytes of the vasa recta, Ang II-induced depletion of the SR Ca2+ store activates store-operated channels. These findings are based on observations that SKF 96365, a nonselective blocker of store-operated channels, produces an identical concentration-dependent blockade of Ang II-selective blocker of store-operated channels, produces an identical concentration-dependent blockade of Ang II-mediated by receptor- and store-operated channels [7]. The molecular isoforms of these channels in pericytes are still not known. While the intracellular release of Ca2+ in response to GPCR activation is well defined, Ca2+ influx in response to agonists in pericytes remains an enigma.

**Cross-Talk between Ca2+ Signalling of the Endothelium and Pericytes**

The microvascular endothelium plays a pivotal role in the control of microvascular tone and capillary recruitment. It is generally accepted that the endothelial synthesis/release of vasoactive mediators such as nitric oxide (NO), prostacyclins and endothelium-derived hyperpolarizing factors is controlled and preceded by changes in the concentration of [Ca2+]i in endothelial cells [79]. Acetylcholine has proven to be the most reliable agonist of endothelial cells and its mechanism of action is well described. Upon binding to muscarinic M3 receptor on the endothelium, phospholipase C activation generates IP3 to release Ca2+ from the endoplasmic reticulum [12, 14, 15, 80, 81]. In turn, the activation of intermediate- and small-conductance Ca2+-activated K+ channels (IKCa and SKCa, respectively) results in hyperpolarization [64–70]. Hyperpolarizing current promotes the closure of VGCC, inhibiting intercellular Ca2+ signalling [15] and causing vasodilation [68, 82–84].

Our recent data reveal that endothelial Ca2+ signalling inhibits [Ca2+]i, waves and oscillations mediated by the IP3R channels in myocytes and pericytes via the L-arginine/NO pathway [15]. These findings are in line with previous results showing that arteriolar dilation by the NO/cGMP/cGKI pathway might involve an inhibition of [Ca2+]i release via IP3R channels [85, 86]. The molecular mechanism by which NO inhibits IP3R is under current investigation. Recently, the PKG substrate, IRAG, has been identified in myocytes and it has been suggested that it blocks IP3R activation [87, 88]. This agrees well with our findings demonstrating that NO by the activation of the cGMP-cGKI pathway elicits a local dilation but fails to initiate a conducting vasodilatory response [79, 89–94].

**Effects of Metabolic Factors**

The control of capillary recruitment and the redistribution of blood flow reside in the terminal arteriolar region of the microcirculation [95–97]. Traditionally, it was thought that Ca2+ signalling in endothelial cells is modulated by metabolic factors, causing local and conducted vasodilation (see above). Recent data suggest that pericytes are metabolically sensitive cells and respond, with the elevation of [Ca2+]i, to several metabolites including lactate [42] and H2O2 [11]. Pericytes may also generate hyperpolarizing signals via the activation of K+ channels and thereby participate in conducted vasodilation [98]. Oxidative stress increases [Ca2+]i predominantly by Ca2+ release from [Ca2+]i stores. This has been shown to be regulated by tyrosine phosphorylation in rat CNS pericytes [11]. In retinal pericytes, lactate induces Ca2+ transients associated with vasoconstriction. It has been suggested that the rise in [Ca2+]i is caused by the reversal of the Na+-Ca2+ exchanger, i.e. it operates in the Ca2+ entry mode. This occurs due to elevation of intracellular Na+ in the endothelium, in response to the influx of H+ and lactate via monocarbox-
ylate transporters. When gap junctions are inhibited, lactate induces relaxation of pericytes [42]; however, since Ca\(^{2+}\) signals under these conditions have not been monitored in either endothelial cells or pericytes, it is not possible to conclude that this is the only mechanism involved.

### Functional Role of Ca\(^{2+}\) Signalling

**Ca\(^{2+}\)-Contraction Coupling in Pericytes**

ET-1 is released by the endothelium and is most likely produced under pathological conditions such as tissue inflammation, sepsis and ischemia reperfusion. ET-1 induces a single, long-lasting Ca\(^{2+}\) spike which activates a strong and prolonged contraction of pericytes in many tissues. The relaxation is so slow that even after complete restoration of the concentration of [Ca\(^{2+}\)]\(_i\), it is delayed for 10–30 min [15] and, in some cases, does not seem to be complete even after >30-min washouts [99]. In this respect, the responses to ET-1 in pericytes are dramatically different from those seen in myocytes, where the dynamics of the Ca\(^{2+}\) signalling and contraction relaxation cycle are very fast [15]. The long-lasting contraction of pericytes induced by ET-1 seems to be the common property of all contractile pericytes and is associated with the complete closure of the precapillary arterioles in the ureter [15] and the vasa recta of the kidney [100] as well as with strong vasoconstriction of all sections of the postcapillary venules in the ureter [15].

Pericytes can also respond to some of the GPCR agonists with a contraction that is controlled by Ca\(^{2+}\) oscillations, e.g. AVP in the rat ureter [15] and Ang II in the vasa recta [18]. The slower Ca\(^{2+}\) dynamics and kinetics of relaxation allow pericytes to generate sustained contraction at a low Ca\(^{2+}\) oscillation frequency (0.03 Hz) whereas for myocytes, a much higher frequency is required (i.e. >0.1 Hz) [15]. The relaxation rate of pericyte contraction is much slower than that of myocytes induced by the same agonist. This observation suggests a difference between myocytes and pericytes in their intrinsic mechanisms for maintaining a contractile force; such mechanisms may be useful in aiding precapillary arterioles, for example, to develop a sustained contraction against the blood pressure. Recent work suggests the role of Ca\(^{2+}\)-sensitizing pathways via Rho kinase or PKC in the regulation of pericyte contractility [71]. Whether precapillary pericytes produce myogenic tone in response to the relatively low blood pressure observed at the terminal branches of precapillary arterioles has still to be studied.

### Summary and Conclusions

In the diverse areas of developmental and vascular biology and pathology, there is currently an explosion of activity in pericycle research. More work is needed to assess all of the possible regulatory agents and cellular mechanisms of Ca\(^{2+}\) signalling and its relation to contraction. Collectively, recent work in this area has given solid support for a contractile function of pericytes and has begun to provide insights into how pericycle function is regulated by hormones, neuromodulators and local tissue factors. Pericytes exhibit a distinct system of Ca\(^{2+}\) signalling and contractility; this needs further investigation. However, the determination of the effects of vasoactive molecules on capillary perfusion awaits the development of a method to internally perfuse the microvascular networks in situ and directly visualize fluorescently labelled blood cells flowing through capillaries ex vivo. New techniques will assist in simultaneous investigation of Ca\(^{2+}\) signals and cell fluxes using multispacial imaging approaches and genetically encoded Ca\(^{2+}\) sensors, which will contribute to a better understanding of how the microvessels are functionally arranged to meet metabolic needs.

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