Calcium Signalling in Pericytes

Theodor Burdyla Lyudmyla Borysova

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

Key Words

Ca²⁺ signalling · Pericytes · Myocytes · Microvascular networks · Arterioles · Capillaries · Venules

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 Ca²⁺ concentration. In this short review, we summarize our results and those of other authors on the contractility of pericytes and their Ca²⁺ signalling. We describe results regarding sources of Ca²⁺ and mechanisms of Ca²⁺ release and Ca²⁺ entry in control of the spatiotemporal characteristics of the Ca²⁺ signals in pericytes.

© 2014 S. Karger AG, Basel

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. Ca²⁺ signalling controls the contractile apparatus of pericytes. The advent of Ca²⁺-sensitive fluorescent dyes, together with video-based imaging and photometric techniques opened the door for the investigation of pericyte Ca²⁺ signalling. The mechanisms of Ca²⁺ entry and Ca²⁺ release, which shape the spatiotemporal profiles of agonist-induced Ca²⁺ signals in pericytes, are under current investigation. Ca²⁺ signalling in cultured peri-

Fig. 1. Morphology of the ureteric microvascular network in situ. **A** Images of precapillary arterioles (i, ii), capillaries (iii) and postcapillary venules (iv). P = Pericytes of precapillary arterioles, capillaries and postcapillary venules, respectively; SMC = smooth muscle cells. **B** Radial sections of arcade and transverse arterioles (i), the precapillary arteriole and capillary (ii) and the postcapillary venule (iii), showing a monolayer of endothelial cells (EC) surrounded by a monolayer of myocytes (SMC) and pericytes (P). **C** Images showing diversity of shapes of pericytes observed in the precapillary arterioles (i), capillaries (ii) and postcapillary venules (iii–v). **D** Images of myocytes (i), precapillary pericytes (ii), capillary pericytes (iii) and postcapillary pericytes (iv) stained with fluorescently labelled phalloidin. Data were reproduced from Borysova et al. [15] with permission.

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 ubiqu-

itous 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. 1Aii, 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 sulphateproteoglycan (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 $[Ca^{2+}]_i$ 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 in-

deed 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 heterogenous 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 polyamine known to inhibit L-type calcium channels [44].

VGCC-Dependent Ca^{2+} Entry Induced by Membrane Depolarization

Membrane potential plays a central role in the control of $[\text{Ca}^{2+}]_i$ 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 $[\text{Ca}^{2+}]_i$ 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]. Vasoconstrictor 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 struc-

tural 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 renalis 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 $[\text{Ca}^{2+}]_i$ 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 $[Ca^{2+}]_i$ 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 $[Ca^{2+}]_i$ [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 vasomotor 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 pacesetters of conducted vasomotion in upstream arterioles. Conversely, hyperpolarizing current 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 $[Ca^{2+}]_i$ 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, (Arg⁸)-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 $[Ca^{2+}]_i$ 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_3R) 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- β 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 non-selective blocker of store-operated channels, produces an identical concentration-dependent blockade of Ang II- and CPA-induced responses in the pericytes of the vasa recta. In CNS pericytes, voltage-independent Ca²⁺ entry is 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 (IK_{Ca} and SK_{Ca}, 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-

plate 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 $[\text{Ca}^{2+}]_i$, 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 pericyte 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 pericyte 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 multispectral 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.

Acknowledgments

This work was supported by the British Heart Foundation PG/12/62/29823.

References

- 1 De Backer D, Ospina-Tascon G, Salgado D, Favory R, Creteur J, Vincent JL: Monitoring the microcirculation in the critically ill patient: current methods and future approaches. *Intensive Care Med* 2010;36:1813–1825.
- 2 Zimmerman KW: Der feinere Bau der Blutcapillaren. *Z Anat Entwicklungsgesch* 1923; 68:29–109.
- 3 Jima T, Zhang JQ, Goto T, Kondo T, Tanaka T: A scanning electron microscopic study of the contraction of vascular wall cells in dog dental. *J Dent Res* 1991;70:1456–1461.
- 4 Higuchi K, Hashizume H, Aizawa Y, Ushiki T: Scanning electron microscopic studies of the vascular smooth muscle cells and pericytes in the rat heart. *Arch Histol Cytol* 2000; 63:115–126.
- 5 Ramachandran E, Frank RN, Kennedy A: Effects of endothelin on cultured bovine retinal microvascular pericytes. *Invest Ophthalmol Vis Sci* 1993;34:586–595.

- 6 Dehouck MP, Vigne P, Torpier G, Breittmayer JP, Cecchelli R, Frelin C: Endothelin-1 as a mediator of endothelial cell-pericyte interactions in bovine brain capillaries. *J Cereb Blood Flow Metab* 1997;17:464–469.
- 7 Kamouchi M, Kitazono T, Ago T, Wakisaka M, Ooboshi H, Ibayashi S, Iida M: Calcium influx pathways in rat CNS pericytes. *Brain Res Mol Brain Res* 2004;126:114–120.
- 8 Kawamura H, Kobayashi M, Li Q, Yamanishi S, Katsumura K, Minami M, Wu DM, Puro DG: Effects of angiotensin II on the pericyte-containing microvasculature of the rat retina. *J Physiol* 2004;561:671–683.
- 9 Wu DM, Kawamura H, Sakagami K, Kobayashi M, Puro DG: Cholinergic regulation of pericyte-containing retinal microvessels. *Am J Physiol Heart Circ Physiol* 2003;284:H2083–H2090.
- 10 McGinty A, Scholfield CN, Liu WH, Anderson P, Hoey DE, Trimble ER: Effect of glucose on endothelin-1-induced calcium transients in cultured bovine retinal pericytes. *J Biol Chem* 1999;274:25250–25253.
- 11 Kamouchi M, Kitazono T, Ago T, Wakisaka M, Kuroda J, Nakamura K, Hagiwara N, Ooboshi H, Ibayashi S, Iida M: Hydrogen peroxide-induced Ca^{2+} responses in CNS pericytes. *Neurosci Lett* 2007;416:12–16.
- 12 Burdya TV, Shmigol A, Eisner DA, Wray S: A new technique for simultaneous and in situ measurements of Ca signals in arteriolar smooth muscle and endothelial cells. *Cell Calcium* 2003;34:27–33.
- 13 Perez JF, Sanderson MJ: The contraction of smooth muscle cells of intrapulmonary arterioles is determined by the frequency of Ca^{2+} oscillations induced by 5-HT and KCl. *J Gen Physiol* 2005;125:555–567.
- 14 Borisova L, Wray S, Eisner DA, Burdya T: How structure, Ca signals, and cellular communications underlie function in precapillary arterioles. *Circ Res* 2009;105:803–810.
- 15 Borysova L, Wray S, Eisner D, Burdya T: How calcium signals in myocytes and pericytes are integrated across in situ microvascular networks and control microvascular tone. *Cell Calcium* 2013;54:163–174.
- 16 Hashitani H, Takano H, Fujita K, Mitsui R, Suzuki H: Functional properties of suburothelial microvessels in the rat bladder. *J Urol* 2011;185:2382–2391.
- 17 Hashitani H, Mitsui R, Shimizu Y, Higashi R, Nakamura K: Functional and morphological properties of pericytes in suburothelial venules of the mouse bladder. *Br J Pharmacol* 2012;167:1723–1736.
- 18 Zhang Q, Cao C, Zhang Z, Wier WG, Edwards A, Pallone TL: Membrane current oscillations in descending vasa recta pericytes. *Am J Physiol Renal Physiol* 2008;294:F656–F666.
- 19 Rucker HK, Wynder HJ, Thomas WE: Cellular mechanisms of CNS pericytes. *Brain Res Bull* 2000;51:363–369.
- 20 Nehls V, Drenckhahn D: Heterogeneity of microvascular pericytes for smooth muscle type alpha-actin. *J Cell Biology* 1991;113:147–154.
- 21 Puro DG: Physiology and pathobiology of the pericyte-containing retinal microvasculature: new developments. *Microcirculation* 2007;14:1–10.
- 22 Gattone VH 2nd, Luft FC, Evan AP: Renal afferent and efferent arterioles of the rabbit. *Am J Physiol* 1984;247(2 Pt 2):F219–F228.
- 23 Shepro D, Morel NM: Pericyte physiology. *FASEB J* 1993;7:1031–1038.
- 24 Majno G, Shea SM, Leventhal M: Endothelial contraction induced by histamine – type mediators. *J Cell Biol* 1969;42:647–672.
- 25 Fujiwara T, Tenkova TI, Kondo M: Wall cytoarchitecture of the rat ciliary process microvasculature revealed with scanning electron microscopy. *Anat Rec* 1999;254:261–268.
- 26 Iijima T, Zhang JQ: Three-dimensional wall structure and the innervations of dental pulp blood vessels. *Microsc Res Tech* 2002;56:32–41.
- 27 DeNofrio D, Hooch TC, Herman IM: Functional sorting of actin isoforms in microvascular pericytes. *J Cell Biol* 1989;109:191–202.
- 28 Nees S, Juchem G, Eberhorn N, Thallmair M, Förch S, Knott M, Senft A, Fischlein T, Reichart B, Weiss DR: Wall structures of myocardial precapillary arterioles and postcapillary venules reexamined and reconstructed in vitro for studies on barrier functions. *Am J Physiol Heart Circ Physiol* 2012;302:H51–H68.
- 29 Condren AB, Kumar A, Mettu P, Liang KJ, Zhao L, Tsai J, Fariss RN, Wong WT: Perivascular mural cells of the mouse choroid demonstrate morphological diversity that is correlated to vasoregulatory function *PLOS ONE* 2013;8:1–13.
- 30 Joyce NC, Haire MF, Palade GE: Contractile proteins in pericytes. I. Immunoperoxidase localization of tropomyosin. *J Cell Biol* 1985;100:1379–1386.
- 31 Joyce NC, DeCamilli P, Boyles J: Pericytes, like vascular smooth muscle cells, are immunocytochemically positive for cyclic GMP-dependent protein kinase. *Microvasc Res* 1984;28:206–219.
- 32 Stark K, Eckart A, Haidari S, Tirniceriu A, Lorenz M, von Brühl ML, Gärtner F, Khandoga AG, Legate KR, Pless R, Hepper I, Lauber K, Walzog B, Massberg S: Capillary and arteriolar pericytes attract innate leukocytes exiting through venules and ‘instruct’ them with pattern-recognition and motility programs. *Nat Immunol* 2013;14:41–51.
- 33 Zhang HR: Scanning electron microscopic study of corrosion casts on retinal and choroidealangioarchitecture in man and animals. *Progr Retinal Eye Res* 1994;13:243–270.
- 34 Pannarale L, Onori P, Ripani M, Gaudio E: Precapillary patterns and perivascular cells in the retinal microvasculature. A scanning electron microscopic study. *J Anat* 1996;188:693–703.
- 35 Mitsui R, Hashitani H: Immunohistochemical characteristics of suburothelial microvasculature in the mouse bladder. *Histochem Cell Biol* 2013;140:189–200.
- 36 Marshall JM, Lloyd J, Mian R: The influence of vasopressin on the arterioles and venules of skeletal muscle of the rat during systemic hypoxia. *J Physiol* 1993;470:473–484.
- 37 Murphy DD, Wagner RC: Differential contractile response of cultured microvascular pericytes to vasoactive agents. *Microcirculation* 1994;1:121–128.
- 38 Sims DE: Recent advances in pericyte biology – implications for health and disease. *Can J Cardiol* 1991;7:431–443.
- 39 Boado RJ, Pardridge WM: Differential expression of alpha-actin mRNA and immunoreactive protein in brain microvascular pericytes and smooth muscle cells. *J Neurosci Res* 1994;39:430–435.
- 40 Bandopadhyay R, Orte C, Lawrenson JG, Reid AR, De Silva S, Allt G: Contractile proteins in pericytes at the blood-brain and blood-retinal barriers. *J Neurocytol* 2001;30:35–44.
- 41 Kawamura H, Sugiyama T, Wu DM, Kobayashi M, Yamanishi S, Katsumura K, Puro DG: ATP: a vasoactive signal in the pericyte-containing microvasculature of the rat retina. *J Physiol* 2003;551:787–799.
- 42 Yamanishi S, Katsumura K, Kobayashi T, Puro DG: Extracellular lactate as a dynamic vasoactive signal in the rat retinal microvasculature. *Am J Physiol Heart Circ Physiol* 2006;290:H925–H934.
- 43 Helbig H, Kornacker S, Berweck S, Stahl F, Lepple-Wienhues A, Wiederholt M: Membrane potentials in retinal capillary pericytes: excitability and effect of vasoactive substances. *Invest Ophthalmol Vis Sci* 1992;33:2105–2112.
- 44 Matsushita K, Fukumoto M, Kobayashi T, Kobayashi M, Ishizaki E, Minami M, Katsumura K, Liao SD, Wu DM, Zhang T, Puro DG: Diabetes-induced inhibition of voltage-dependent calcium channels in the retinal microvasculature: role of spermine. *Invest Ophthalmol Vis Sci* 2010;51:5979–5990.
- 45 Zhang Z, Rhinehart K, Pallone TL: Membrane potential controls calcium entry into descending vasa recta pericytes. *Am J Physiol* 2002;283:R949–R957.
- 46 Sakagami K, Wu DM, Puro DG: Physiology of rat retinal pericytes: modulation of ion channel activity by serum-derived molecules. *J Physiol* 1999;521:637–650.
- 47 Ishizaki E, Fukumoto M, Puro DG: Functional K(ATP) channels in the rat retinal microvasculature: topographical distribution, redox regulation, spermine modulation and diabetic alteration. *J Physiol* 2009;587:2233–2253.
- 48 Zhang Z, Cao C, Lee-Kwon W, Pallone TL: Descending vasa recta pericytes express voltage operated Na^{+} conductance in the rat. *J Physiol* 2005;567:445–457.

- 49 Lee-Kwon W, Wade JB, Zhang Z, Pallone TL, Weinman EJ: Expression of TRPC4 channel protein that interacts with NHERF-2 in rat descending vasa recta. *Am J Physiol Cell Physiol* 2005;288:C942–C949.
- 50 Beach JM, McGahren ED, Duling BR: Capillaries and arterioles are electrically coupled in hamster cheek pouch. *Am J Physiol Heart Circ Physiol* 1998;275:H1489–H1496.
- 51 Berg BR, Cohen KD, Sarelius IH: Direct coupling between blood flow and metabolism at the capillary level in striated muscle. *Am J Physiol Heart Circ Physiol* 1997;272:H2693–H2700.
- 52 Song H, Tyml K: Evidence for sensing and integration of biological signals by the capillary network. *Am J Physiol Heart Circ Physiol* 1993;265:H1235–H1242.
- 53 Mitchell D, Tyml K: Nitric oxide release in rat skeletal muscle capillary. *Am J Physiol* 1996;270(5 Pt 2):H1696–H1703.
- 54 De Wit C, Roos F, Bolz SS, Kirchhoff S, Krüger O, Willecke K, Pohl U: Impaired conduction of vasodilation along arterioles in connexin 40-deficient mice. *Circ Res* 2000;86:649–655.
- 55 Dora KA, Xia J, Duling BR: Endothelial cell signalling during conducted vasomotor responses. *Am J Physiol Heart Circ Physiol* 2003;285:H119–H126.
- 56 Segal SS, Duling BR: Flow control among microvessels coordinated by intercellular conduction. *Science* 1986;234:868–870.
- 57 Emerson GG, Segal SS: Endothelial cell pathway for conduction of hyperpolarization and vasodilation along hamster feed artery. *Circ Res* 2000;86:94–100.
- 58 Oku H, Kodama T, Sakagami Wu DM, Minami M, Kawamura H, Puro DG: Electrotonic transmission within pericyte-containing retinal microvessels. *Microcirculation* 2006;13:353–363.
- 59 Matsushita K, Puro DG: Topographical heterogeneity of KIR currents in pericyte-containing microvessels of the rat retina: effect of diabetes. *J Physiol* 2006;573:483–495.
- 60 Zhang T, Wu DM, Xu GZ, Puro DG: The electrotonic architecture of the retinal microvasculature: modulation by angiotensin II. *J Physiol* 2011;589:2383–2399.
- 61 Wu DM, Minami M, Kawamura H, Puro DG: Electrotonic transmission within pericyte-containing retinal microvessels. *Microcirculation* 2006;13:353–363.
- 62 Mogensen C, Bergner B, Wallner S, Ritter A, D'Avis S, Ninichuk V, Kameritsch P, Gloe T, Nagel W, Pohl U: Isolation and functional characterization of pericytes derived from hamster skeletal muscle. *Acta Physiol* 2011;201:413–426.
- 63 Zhang Z, Payne K, Cao C, Pallone TL: Mural propagation of descending vasa recta responses to mechanical stimulation. *Am J Physiol Renal Physiol* 2013;305:F286–F294.
- 64 Busse R, Fichtner H, Luckhoff A, Kohlhardt M: Hyperpolarization and increased free calcium in acetylcholine-stimulated endothelial cells. *Am J Physiol Heart Circ Physiol* 1988;255:H965–H969.
- 65 Olesen SP, Davies PF, Clapham DE: Muscarinic activated K⁺ current in bovine aortic endothelial cells. *Circ Res* 1988;62:1059–1064.
- 66 Sakai T: Acetylcholine induces Ca-dependent K currents in rabbit endothelial cells. *Jpn J Pharmacol* 1990;53:235–246.
- 67 Jackson WF: Potassium channels in the peripheral microcirculation. *Microcirculation* 2005;12:113–127.
- 68 Ledoux J, Werner ME, Brayden JE, Nelson MT: Calcium-activated potassium channels and the regulation of vascular tone. *Physiology* 2006;21:69–78.
- 69 Domeier TL, Segal SS: Electromechanical and pharmacomechanical signalling pathways for conducted vasodilation along endothelium of hamster feed arteries. *J Physiol* 2007;579:175–186.
- 70 Wölfe SE, Schmidt VJ, Hoyer J, Köhler R, De Wit C: Prominent role of KCa3.1 in endothelium-derived hyperpolarizing factor-type dilations and conducted responses in the microcirculation in vivo. *Cardiovasc Res* 2009;82:476–483.
- 71 Kutcher ME, Kolyada AY, Surks HK, Herman IM: Pericyte Rho GTPase mediates both pericyte contractile phenotype and capillary endothelial growth state. *Am J Pathol* 2007;171:693–701.
- 72 Berridge MJ: Inositol trisphosphate and calcium signalling. *Nature* 1993;361:315–325.
- 73 Scholfield CN, Curtis TM: Heterogeneity in cytosolic calcium regulation among different microvascular smooth muscle cells of the rat retina. *Microvasc Res* 2000;59:233–242.
- 74 Chakravarthy U, Gardiner TA, Archer DB, Trimble ER: The effect of endothelin 1 on the retinal microvascular pericyte. *Microvasc Res* 1992;43:241–254.
- 75 Rhinehart K, Zhang Z, Pallone TL: Ca²⁺ signaling and membrane potential in descending vasa recta pericytes and endothelia. *Am J Physiol* 2002;283:F852–F860.
- 76 Tumelty J, Hinds K, Bankhead P, McGeown NJ, Scholfield C, Curtis TM, McGeown JG: Endothelin 1 stimulates Ca²⁺ sparks and oscillations in retinal arteriolar myocytes via IP₃R and RyR-dependent Ca²⁺ release. *Invest Ophthalmol Vis Sci* 2011;52:3874–3879.
- 77 Iino M, Kasai H, Yamazawa T: Visualization of neural control of intracellular Ca²⁺ concentration in single vascular smooth muscle cells in situ. *EMBO J* 1994;13:5026–5031.
- 78 Pallone TL, Huang JM: Control of descending vasa recta pericyte membrane potential by angiotensin II. *Am J Physiol* 2002;282:F1064–F1074.
- 79 Tallini YN, Brekke JF, Shui B, Doran R, Hwang SM, Nakai J, Salama G, Segal SS, Kotlikoff MI: Propagated endothelial Ca²⁺ waves and arteriolar dilation in vivo: measurements in Cx40BAC GCaMP2 transgenic mice. *Circ Res* 2007;101:1300–1309.
- 80 Himmel HM, Whorton AR, Strauss HC: Intracellular calcium, currents, and stimulus-response coupling in endothelial cells. *Hypertension* 1993;21:112–127.
- 81 Danthuluri NR, Cybulsky MI, Brock TA: Ach induced calcium transients in primary cultures of rabbit aortic endothelial cells. *Am J Physiol Heart Circ Physiol* 1988;255:H1549–H1553.
- 82 Nelson MT, Patlak JB, Worley JF, Standen NB: Calcium channels, potassium channels, and voltage dependence of arterial smooth muscle tone. *Am J Physiol* 1990;259:C3–C18.
- 83 Bolz SS, De Wit C, Pohl U: Endothelium-derived hyperpolarizing factor but not NO reduces smooth muscle Ca²⁺ during acetylcholine-induced dilation of microvessels. *Br J Pharmacol* 1999;128:124–134.
- 84 Ungvari Z, Csizsar A, Koller A: Increases in endothelial Ca(2+) activate K(Ca) channels and elicit EDHF-type arteriolar dilation via gap junctions. *Am J Physiol Heart Circ Physiol* 2002;282:H1760–H1767.
- 85 Schlossmann J, Ammendola A, Ashman K, Zong XG, Huber A, Neubauer G, Wang GX, Allescher HD, Korth M, Wilm M, Hofmann F, Ruth P: Regulation of intracellular calcium by a signalling complex of IRAG, IP₃ receptor and cGMP kinase I beta. *Nature* 2000;404:197–201.
- 86 Feil R, Gappa N, Rutz M, Schlossmann J, Rose CR, Konnerth A, Brummer S, Kuhbandner S, Hofmann F: Functional reconstitution of vascular smooth muscle cells with cGMP-dependent protein kinase I isoforms. *Circ Res* 2002;90:1080–1086.
- 87 Carvajal JA, Germain AM, Huidobro-Toro JP, Weiner CP: Molecular mechanism of cGMP-mediated smooth muscle relaxation. *J Cell Physiol* 2000;184:409–420.
- 88 Ammendola A, Geiselhöringer A, Hofmann F, Schlossmann J: Molecular determinants of the interaction between the inositol 1,4,5-trisphosphate receptor-associated cGMP kinase substrate (IRAG) and cGMP kinase Ibeta. *J Biol Chem* 2001;276:24153–24159.
- 89 Hungerford JE, Sessa WC, Segal SS: Vasomotor control in arterioles of the mouse cremaster muscle. *FASEB J* 2000;14:197–207.
- 90 Doyle MP, Duling BR: Acetylcholine induces conducted vasodilation by nitric oxide-dependent and -independent mechanisms. *Am J Physiol* 1997;272:H1364–H1371.
- 91 Koeppen M, Feil R, Siegl D, Feil S, Hofmann F, Pohl U, De Wit C: cGMP-dependent protein kinase mediates NO but not acetylcholine-induced dilations in resistance vessels in vivo. *Hypertension* 2004;44:952–955.

- 92 Delashaw JB, Duling BR: Heterogeneity in conducted arteriolar vasomotor response is agonist dependent. *Am J Physiol Heart Circ Physiol* 1991;260:H1276–H1282.
- 93 Kurjiaka DT, Segal SS: Conducted vasodilation elevates flow in arteriole networks of hamster striated muscle. *Am J Physiol Heart Circ Physiol* 1995;269:H1723–H1728.
- 94 Hoepfl B, Rodenwaldt B, Pohl U, De Wit C: EDHF, but not NO or prostaglandins, is critical to evoke a conducted dilation upon ACh in hamster arterioles. *Am J Physiol Heart Circ Physiol* 2002;283:H996–H1004.
- 95 Klitzman B, Damon DN, Gorczynski RJ, Duling BR: Augmented tissue oxygen supply during striated muscle contraction in the hamster. Relative contributions of capillary recruitment, functional dilation, and reduced tissue PO₂. *Circ Res* 1982;51:711–721.
- 96 Lindbom L, Arfors KE: Non-homogeneous blood flow distribution in the rabbit tenuissimus muscle. Differential control of total blood flow and capillary perfusion. *Acta Physiol Scand* 1984;122:225–233.
- 97 Sarelius IH: Cell flow path influences transit time through striated muscle capillaries. *Am J Physiol* 1986;250(6 Pt 2):H899–H907.
- 98 Yamanishi S, Katsumura K, Kobayashi T, Puro DG: Extracellular lactate as a dynamic vasoactive signal in the rat retinal microvasculature. *Am J Physiol* 2006;290:H925–H934.
- 99 Silldorff EP, Yang S, Pallone TL: Prostaglandin E₂ abrogates endothelin-induced vasoconstriction in renal outer medullary descending vasa recta of the rat. *J Clin Invest* 1995;95:2734–2740.
- 100 Pallone TL, Zhang Z, Rhinehart K: Physiology of the renal medullary microcirculation. *Am J Physiol Renal Physiol* 2003;284:F253–F266.