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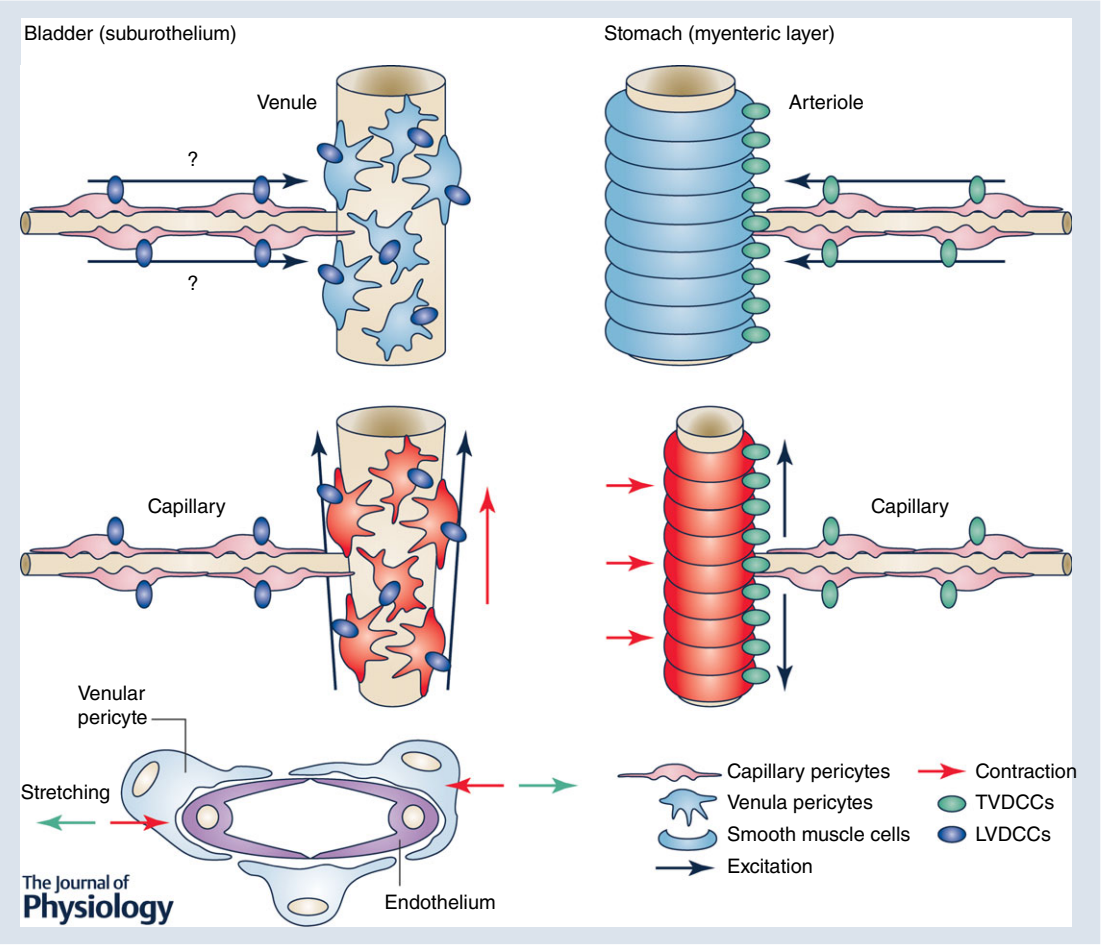
TOPICAL REVIEW

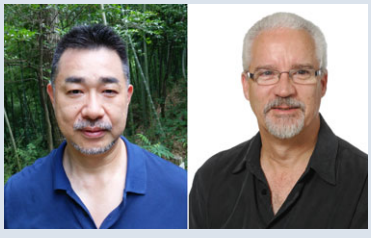
Spontaneous activity in the microvasculature of visceral organs: role of pericytes and voltage-dependent Ca^{2+} channels

Hikaru Hashitani[1] and Richard J. Lang[2]

[1] *Department of Cell Physiology, Graduate School of Medical Sciences, Nagoya City University, Nagoya, Japan*
[2] *Department of Physiology, School of Biomedical Sciences, Monash University, Clayton, Victoria, 3800, Australia*



Hikaru Hashitani has been Professor of Cell Physiology at Nagoya City University since 2010. He received his medical degree from Kyushu University, completed his PhD at Nagoya City University, and then studied at Melbourne University and University of Oxford. His research interests have primarily focused on cellular mechanisms underlying spontaneous electrical and calcium signalling in smooth muscle of the genitourinary, gastrointestinal and microvascular systems. **Richard Lang** received his PhD Monash University in 1979. He was an MRC Research Officer and Fellow 1978–1986 in the Department of Pharmacology, St George's Hospital Medical School, London. He returned to Monash University as a Vice-Chancellor's Research Fellow (1986–1987), an NHRMC Senior Research Officer (1988–1990) and a Senior Research Fellow (1991–). Throughout, he has been interested in the role membrane channels and calcium play in pacemaker and tone generation in both gastrointestinal and urogenital organs.



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Abstract The microvasculature plays a primary role in the interchange of substances between tissues and the circulation. In visceral organs that undergo considerable distension upon filling, the microvasculature appears to display intrinsic contractile properties to maintain their flow. Submucosal venules in the bladder or gastrointestinal tract generate rhythmic spontaneous phasic constrictions and associated Ca^{2+} transients. These events are initiated within either venular pericytes or smooth muscle cells (SMCs) arising from spontaneous Ca^{2+} release from the sarcoplasmic reticulum (SR) and the opening of Ca^{2+}-activated chloride channels (CaCCs) that trigger Ca^{2+} influx through L-type voltage-dependent Ca^{2+} channels (VDCCs). L-type VDCCs also play a critical role in maintaining synchrony within the contractile mural cells. In the stomach myenteric layer, spontaneous Ca^{2+} transients originating in capillary pericytes appear to spread to their neighbouring arteriolar SMCs. Capillary Ca^{2+} transients primarily rely on SR Ca^{2+} release, but also require Ca^{2+} influx through T-type VDCCs for their synchrony. The opening of T-type VDCCs also contribute to the propagation of Ca^{2+} transients into SMCs. In visceral microvasculature, pericytes act as either spontaneously active contractile machinery of the venules or as pacemaker cells generating synchronous Ca^{2+} transients that drive spontaneous contractions in upstream arterioles. Thus pericytes play different roles in different vascular beds in a manner that may well depend on the selective expression of T-type and L-type Ca^{2+} channels.

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Corresponding author H. Hashitani: Department of Cell Physiology, Nagoya City University Graduate School of Medical Sciences, Nagoya 467–8601, Japan. Email: hasitani@med.nagoya-cu.ac.jp

Abstract figure legend Pericytes play different roles in different microcirculatory beds In the bladder suburothelium (left), 'non contractile' capillary pericytes (CPCs) may generate 'spreading' excitation to drive venular pericytes (VPCs; upper). Excitation of VPCs upon the opening of L-type voltage-dependent Ca^{2+} channels (LVDCCs) spread within a VPC network to generate 'peristaltic' vasoconstrictions (middle). Contractions of VPCs also work against stretching during storage phase to prevent venular collapse (lower). In the myenteric layer of the stomach (right), 'non contractile' CPCs generate 'spreading' excitation upon the opening of T-type voltage-dependent Ca^{2+} channels (TVDCCs) to drive smooth muscle cells (SMCs; upper). Excitation of SMCs upon the opening of TVDCCs spread within a SMC network to generate 'synchronous' vasoconstrictions (lower). Cells in red indicate excited and contracted. Cells in light blue indicate in resting state. Cells in orange indicate excited but non-contractile.

Abbreviations α-SMA, alpha smooth muscle actin; ACh, acetylcholine; CaCC, Ca^{2+}-activated chloride channel; CGRP, calcitonin gene-related peptide; EFS, electrical field stimulation; CICR, Ca^{2+}-induced Ca^{2+} release; GI, gastrointestinal; $InsP_3$, inositol trisphosphate; ICC, interstitial cells of Cajal; NCX, sodium–calcium exchangers; NO, nitric oxide; PKG, protein kinase G; PLC, phospholipase C; SERCA, sarco-endoplasmic reticulum Ca^{2+}-ATPase; sGC, soluble guanylate cyclase; SMC, smooth muscle cell; SOC, store-operated Ca^{2+} entry channel; SR, sarcoplasmic reticulum; VDCC, voltage-dependent Ca^{2+} channel.

Introduction

The microvasculature consisting of precapillary arterioles, capillaries and postcapillary venules regulates the transport of nutrients to tissues and removal of their excreta. Arterioles control the blood flow into the tissues to meet the demands of individual organs, while transfer of substances predominately occurs across the wall of the capillaries. Since capillary filtration and reabsorption is a function of the hydrostatic pressure that is determined by arteriolar and venular pressures and the ratio of post-to-precapillary resistance, the different components of the microvasculature play a critical role in regulating the microcirculation. The relative importance of these different units may vary amongst organs depending on extravascular factors, e.g. extraluminal tissue pressure and stretch or compression associated with tissue volume changes.

In visceral organs that undergo considerable wall distention or rises in the luminal pressure upon filling, e.g. urinary bladder (Hashitani *et al.* 2011, 2012; Shimizu *et al.* 2014), stomach (Mitsui & Hashitani, 2015*a*; Hashitani *et al.* 2015) and colon (Mitsui *et al.* 2013), the microvasculature appears to display intrinsic contractile properties to maintain their flow. Previous studies on the contractile properties of the microvasculature in visceral organs have mostly focused on the arterioles, particularly those in the gastrointestinal tract (Hirst, 1977; Kotecha & Neild, 1995). The properties of the mural cells, i.e. SMCs and/or pericytes, in capillaries and venules have been less explored. Since even small rises in the intraluminal pressure and/or associated organ wall distension may

diminish blood flow in capillaries or venules more readily than in arterioles, the intrinsic contractile properties of these microvessels should be further investigated. In addition, since contractility of microvasculature is regulated by both neural activity and intrinsic 'myogenic' activity, it is reasonable to assume that spontaneous contractile activity of microvasculature in visceral organs plays a fundamental role in the maintenance of the microcirculation.

Arterioles or venules are surrounded by densely packed vascular SMCs, while capillaries and pre- and post-capillary microvessles are covered by morphologically distinct pericytes. Pericytes in precapillary arterioles and postcapillary venules express α-smooth muscle actin (α-SMA) and are contractile, while capillary pericytes are often negative for α-SMA and non-contractile (Burdyga & Borysova, 2014). However, the role of pericytes in regulating capillary blood flow has been recently revealed (Peppiatt *et al.* 2006; Femández-Klett *et al.* 2010; Hall *et al.* 2014). In several vascular beds, capillary pericytes have been shown to display Ca^{2+} transients, membrane depolarisations and contractile responses upon stimulation with neurotransmitters or humoral substances, e.g. noradrenaline (Hall *et al.* 2014), acetylcholine (Wu *et al.* 2003), angiotensin-II (Zhang *et al.* 2008) or endothelin-1 (Borysova *et al.* 2013). Pericyte properties have been investigated mostly in the cerebral and retinal microvasculature (Peppiatt *et al.* 2006; Femández-Klett *et al.* 2010; Hall *et al.* 2014), only a few studies have been made in visceral organs (Borysova *et al.* 2013). In particular, it has not been established whether capillary pericytes develop any spontaneous activity that contributes to the regulation of capillary blood blow, or whether venular pericytes in visceral organs exhibit any spontaneous electrical, Ca^{2+} and contractile activity.

This article summarises recent examinations of how spontaneous activity in the microvasculature of visceral organs can be modulated by neurohumoral substances. Spontaneous phasic constrictions resulting from spontaneous Ca^{2+} transients and associated depolarisations appear to be primarily initiated by Ca^{2+} release from SR within mural cells. Different types of VDCCs contribute to not only the amplification of these Ca^{2+} transients but also to the synchrony amongst mural cells. Importantly, pericytes may act as originators for spontaneous activity in the microvasculature tree.

Suburothelial venule of the bladder

Spontaneous venular constrictions. The urinary bladder is capable of accommodating large volumes of urine with remarkably little increase in intravesical pressure. Since intravesical pressure during bladder filling does not exceed capillary pressure, normal bladders maintain their circulation, so that the blood supply only transiently drops during voiding (Greenland & Brading, 1996). Blood vessels in the bladder wall are characterized by their winding arrangement, which prevents them from being stretched in their longitudinal direction during the filling phase (Sarma, 1981). Thus, they are capable of maintaining their diameter so that the resistance against blood flow is not increased.

Besides this structural characteristic, suburothelial venules in the bladder of rat (Hashitani *et al.* 2011; Shimizu *et al.* 2014) and mouse (Hashitani *et al.* 2012) develop spontaneous phasic constrictions, suggesting that the venules actively contribute to the regulation of the microcirculation. In contrast, suburothelial arterioles are not spontaneously active in both species, and their constrictions are exclusively mediated by sympathetic transmission.

Contractile mural cells. In the rat bladder, venular constrictions predominately arise through contractions of circumferentially arranged 'slender' SMCs. However, stellate-shaped pericytes also exhibited spontaneous Ca^{2+} transients and constrictions. Where these SMCs and pericytes are co-localised, they display synchronous Ca^{2+} transients, suggesting that they are coupled with each other. The contractile mural cells generating spontaneous Ca^{2+} transients in the mouse bladder are stellate-shaped pericytes, while spindle-shaped SMCs are distributed only in the proximal, larger venules. Consistent with this vigorous contractility of the venular pericytes and SMCs, immunohistochemistry reveals that both mural cell types express α-SMA. Interestingly, venular pericytes are not immunoreactive against the pericyte marker NG2 (Ozerdem *et al.* 2001), while pericytes in capillaries and arteriolar SMCs both express NG2 (Mitsui & Hashitani, 2013). The morphological characteristics of suburothelial venular pericytes are consistent with those of other vascular beds, having stellate-shaped cell bodies with extensive processes (Higuchi *et al.* 2000; Borysova *et al.* 2013) and a $NG2^{-}$, α-SMA^{+} phenotype (see Burdyga & Borysova, 2014). Pericytes have been considered to be multipotent precursors for several different cell types, including SMCs (Hirschi & D'Amore, 1996) and thus there seems to be a transition from pericyte-enveloped to SMC-enveloped venules, presumably to meet functional demands.

Role of L-type VDCCs. Spontaneous action potentials with a slow upstroke and decay precede phasic constrictions. Blockers for L-type voltage-dependent Ca^{2+} channels (LVDCCs) suppress action potentials and associated constrictions leaving small fluctuations of the membrane potential, indicating that electro-mechanical coupling upon the opening of LVDCCs plays a fundamental role in venular constriction. The predominant role

of LVDCCs in Ca^{2+} influx of SMCs is well established. Ca^{2+} currents arising from the opening of LVDCCs have also been demonstrated in freshly isolated retinal pericytes of the rat (Sakagami *et al.* 1999).

Blockers of LVDCCs reduce the amplitude of venule spontaneous Ca^{2+} transients and also disrupts their synchrony amongst the contractile mural cells, indicating that LVDCCs play a critical role in maintaining multicellular coupling (Fig. 1; Hashitani *et al.* 2011, 2012). Because of the regenerative nature of LVDCC activation, spontaneous action potentials generated in any mural cell within their network spreads to 'electrically-coupled' neighbouring cells irrespective of their length constant. Pericytes in the suburotheial venules appear to make close appositions with themselves as well as the endothelium via gap junctions (Cuevas *et al.* 1984). Since endothelial cells are well coupled to each other and act as a low resistance path of electrical transmission (Yamamoto *et al.* 2001), pericyte depolarisation may even be effectively transmitted to electrically remote pericytes via the endothelium.

Role of SR Ca^{2+} release. After the blockade of LVDCCs, both pericytes and SMCs are capable of generating asynchronous spontaneous Ca^{2+} transients. These residual Ca^{2+} transients are abolished by CPA, an inhibitor for sarco-endoplasmic reticulum Ca^{2+}-ATPase (SERCA), 2-APB, a blocker for inositol trisphosphate ($InsP_3$)-induced Ca^{2+} release, or U73122, a phospholipase C (PLC) inhibitor, but not ryanodine, suggesting a primary role of $InsP_3$-induced Ca^{2+} release from SR in their generation. Spontaneous SR Ca^{2+} release opens Ca^{2+}-activated chloride channels (CaCCs), the resultant depolarization triggering the opening of LVDCCs and associated Ca^{2+} influx to cause venular constrictions (Fig. 2). Such a primary role of CaCCs in electro-mechanical coupling in pericytes has been demonstrated in other vascular beds (Sakagami *et al.* 1999; Zhang *et al.* 2008).

SR Ca^{2+} stores have been shown to function through $InsP_3$-induced Ca^{2+} release in interstitial cells of Cajal (ICC) of the GI tract (Suzuki *et al.* 2000; van Helden *et al.* 2000), and lymphatic SMCs (van Helden, 1993; Imtiaz *et al.* 2007). In comparison, some blood vessels and interstitial cells of the urethra and renal pelvis have Ca^{2+} stores that function through both ryanodine- and $InsP_3$-receptors (Sergeant *et al.* 2001; Haddock & Hill, 2005; Lang *et al.* 2007).

Besides the periodical cycle of SR Ca^{2+} handling, Ca^{2+} influx via store-operated Ca^{2+} entry channels (SOCs) but not sodium calcium exchangers (NCXs) is required in maintaining spontaneous activity of the sub-urothelial venules of the bladder. This is in contrast to the pacemaking mechanism of interstitial cells of the urethra that relies on Ca^{2+} influx via NCXs but not SOCs (Bradley *et al.* 2005, 2006). In addition, Ca^{2+} handling by mitochondria may also be involved in the operation of cytosolic Ca^{2+} oscillators underlying spontaneous venular constrictions, as has been demonstrated in ICC in the GI tract (Ward *et al.* 2000), interstitial cells in the urethra (Sergeant *et al.* 2008) or atypical smooth muscle cells in the renal pelvis (Hashitani *et al.* 2009).

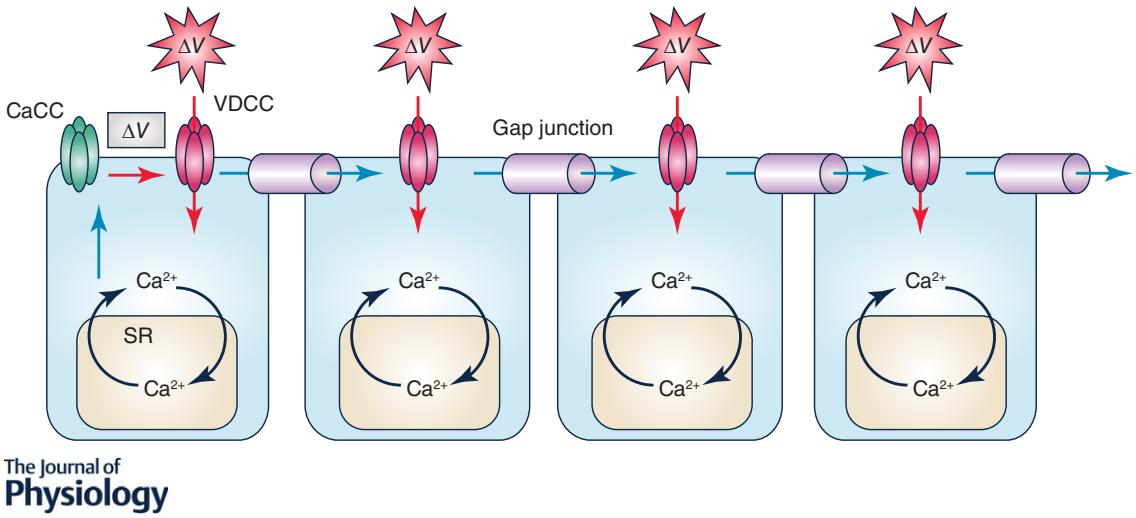

Figure 1. Role of VDCCs in 'coupled' oscillators in the microvasculature
Mural cells in the microvasculature are electrically coupled via gap junctions. Adjacent monolayers of endothelial cells may act as a low resistant pathway for intercellular communications. Spontaneous SR Ca^{2+} release in a mural cell causes the opening of Ca^{2+} activated Cl^- channels (CaCCs) and membrane depolarization (ΔV in grey box). This depolarisation triggers the opening of either L- or T-type VDCCs to cause the firing of 'regenerative' action potentials (ΔV in red exploded polygons). These regenerative action potentials spread to coupled mural cells, and the Ca^{2+} influx via VDCCs triggers Ca^{2+}-induced Ca^{2+} release (CICR).

Modulation of spontaneous activity. Spontaneous constrictions of the suburothelial venules are accelerated upon the activation of α-adrenoceptors by neutrally released noradrenaline (Shimizu *et al.* 2014). After the blockade of α-adrenocetors, sympathetic nerve stimulation abolishes spontaneous constrictions or reduces their frequency upon the activation β-adrenoceptors. This inhibition of spontaneous constrictions is largely diminished upon blockade of nitric oxide (NO) production, suggesting that β-adrenoceptors stimulate NO production presumably within the endothelium. Since suburothelial venules are surrounded by a monolayer of interconnecting stellate-shaped pericytes connecting via thin processes with each other (Hashitani *et al.* 2012), neurally released noradrenaline may readily have access to endothelial receptors through these inter-process spaces. Alpha-adrenoceptors are coupled to Gq/11 proteins which activates PLC to produce $InsP_3$, while cGMP, a second messenger of NO, is known to inhibit $InsP_3$R-induced Ca^{2+} release (Kannan *et al.* 1997). Therefore, the adrenoceptors-mediated changes in the frequency of spontaneous constrictions results from the modulation of $InsP_3$R-induced Ca^{2+} release (Fig. 2). In pericytes of several vascular beds, binding of neurohumoral substances to G-protein coupled receptors, including α-adrenoceptors, is reported to induce $InsP_3$-induced Ca^{2+} release from SR (Zhang *et al.* 2008; Borysova *et al.* 2013).

Blockade of NO production in the bladder increases the frequency of spontaneous venular constrictions. Pericytes in other vascular beds express soluble guanylate cyclase (sGC) and protein kinase G (PKG), and NO donor-induced dilatation is prevented by sGC inhibition (Hamilton *et al.* 2010). Electron microscopy reveals that numerous fusions or close appositions of pericyte and endothelial membranes, especially in their processes; although gap junctions or other junctional structures are not evident (Hashitani *et al.* 2012). Therefore, spontaneous Ca^{2+} transients in pericytes may well spread to the endothelium to increase $[Ca^{2+}]_i$ resulting in the production and release of NO (Fig. 2). In the descending vasa recta of the rat, angiotensin II induces similar depolarisations in pericytes and the endothelium, suggesting electrical coupling via myoendothelial gap junctions (Zhang *et al.* 2014). However, corresponding increases in $[Ca^{2+}]_i$ in pericytes did not spread to the endothelium (Zhang *et al.* 2008). Thus, it may be possible that pericyte depolarisation transmits to the endothelium to activate T-type VDCCs (TVDCCs) to cause Ca^{2+}-dependent NO production as suggested in other vascular preparations (Kuo *et al.* 2011). Nevertheless, the interaction between pericytes and the endothelium in the suburothelial venules may act as self-limiting mechanism to prevent excessive venular constrictions.

Submucosal venule of the GI tract

Spontaneous activity. Submucosal venules of gastric antrum (Mitsui & Hashitani, 2015*a*) and distal colon (Mitsui *et al.* 2013) also develop spontaneous phasic constrictions. Unlike the suburothelial venules of the bladder, the contractile mural cells in the submucosal venules of the stomach and colon are circumferentially arranged SMCs, not stellate-shaped

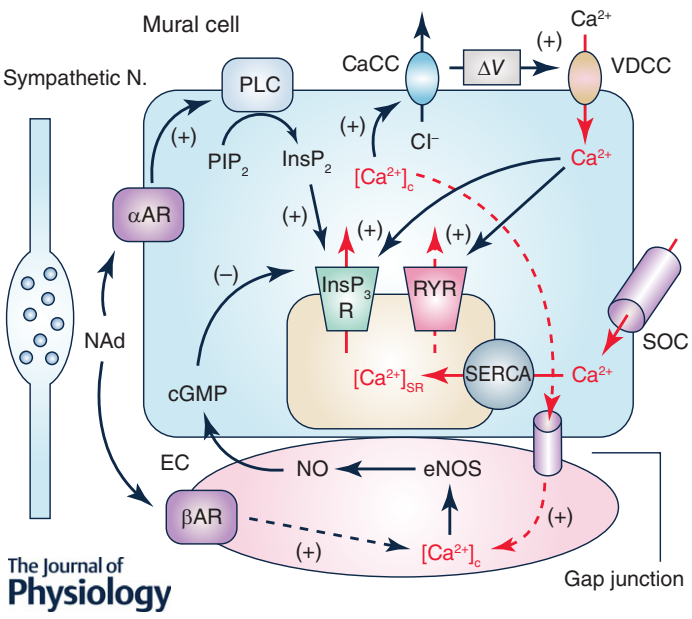

Figure 2. Mechanisms of cytosolic Ca^{2+} oscillator in mural cells of the microvasculature
Mural cells, i.e. pericytes and venular SMCs, operate a 'cytosolic Ca^{2+} oscillator' primarily arising from Ca^{2+} release from SR via inositol trisphosphate receptors ($InsP_3$Rs). Ryanodine receptor (RYRs) may also contribute to Ca^{2+}-induced Ca^{2+} release (CICR) from SR. Increased cytosolic Ca^{2+} concentration ($[Ca^{2+}]_c$) causes openings of Ca^{2+}-activated Cl^- channels (CaCCs) to depolarise the membrane. The depolarisation triggers the opening of voltage-dependent Ca^{2+} channels (VDCCs) to induce Ca^{2+} influx that may be amplified by CICR. Reduction in $[Ca^{2+}]_{SR}$ triggers Ca^{2+} influx via store-operated Ca^{2+} channels (SOCs) that will be taken up via sarco-endoplasmic reticulum Ca^{2+}-ATPase (SERCA) to refill SR. Noradrenaline (NAd) released from sympathetic nerves acting on α-adrenoceptors (αARs) stimulate the production of $InsP_3$ to facilitate SR Ca^{2+} release via $InsP_3$R. NAd binding to β-adrenoceptors (βARs) on endothelial cells stimulates the production of NO. NO increases cGMP in mural cells to inhibit SR Ca^{2+} release via $InsP_3$R. Arrows and dotted arrows in red indicate flows of Ca^{2+}. Dashed arrows indicate proposed or unidentified signalling pathways. eNOS, endothelial NO synthase; EC, endothelium; ΔV, membrane depolarization; PLC, phospholipase C.

pericytes. Stellate-shaped pericytes in post-capillary venules of the gastric antrum have recently been found to exhibit properties similar to those of venular SMCs (Mitsui & Hashitani, 2015*b*). Venules in the wall of in the stomach and colon are likely to be chronically stretched and/or compressed during accommodation of the luminal content. Thus, rhythmic constrictions of these venules may be beneficial in preventing blood stagnation in venules and/or capillaries to maintain submucosal microcirculation.

Intracellular mechanisms. Blockade of LVDCCs suppresses spontaneous Ca^{2+} transients and also disrupts synchrony amongst the venular SMCs of the stomach submucosa (Mitsui & Hashitani, 2015*a*), indicating LVDCCs play a critical role in promoting intercellular coupling (Fig. 1). As a consequence, blockers for LVDCCs abolish spontaneous venular constrictions and causes dilatation. Thus, LVDCCs contribute to not only the generation of phasic constrictions but also basal tone.

Spontaneous Ca^{2+} transients and associated constrictions are also blocked upon inhibition of SERCA or $InsP_3$-induced Ca^{2+} release, but not Ca^{2+}-induced Ca^{2+} release (CICR) via ryanodine receptors, indicating that mechanisms underlying spontaneous activity of the submucosal venules are basically similar to those of suburothelial venules of the bladder (Fig. 2). In addition, blockers for CaCCs channels suppress phasic constrictions associated with a dilatation, suggesting that depolarisations resulting from the opening of CaCCs are required to activate LVDCCs.

Spontaneous venular constrictions are accelerated upon blockade of NO production, while acetylcholine (ACh) suppresses constrictions in an NO-dependent manner, suggesting that endothelium-derived NO increases cGMP in venular SMCs to inhibit $InsP_3$-induced Ca^{2+} release and cytosolic Ca^{2+} oscillations (Fig. 2).

Neuronal modulation. Noradrenaline released from sympathetic nerves induces long-lasting vasoconstriction in GI venules that is blocked by phentolamine, indicating that neutrally mediated venular constriction is exclusively mediated upon the activation of α-adrenoceptors. Thus, unlike arteriolar constrictions, purinergic transmission is not involved in nerve-mediated venular constriction. Consistent with this, immunoreactivity of P2X purinoceptors is detected in the arterioles, but not in neighbouring venules. Spontaneous venular constrictions are suppressed upon activation of primary afferents with capsaicin which is attenuated by a calcitonin gene-related peptide (CGRP) receptor antagonist. Immunohistochemical data reveal that both tyrosine hydroxylase-positive sympathetic nerves and CGRP-expressing nerves project to venules.

Myenteric microvasculatur of the stomach

In the gastrointestinal tract, the myenteric layer, comprising an extensive nerve plexus and networks of ICC and platelet-derived growth factor receptor α (PDGFRα)-positive (PDGFRα^+) fibroblast-like interstitial cells, plays a central role in regulating gastrointestinal motility (Sanders *et al.* 2014). Despite the importance of this layer, previous studies have primarily focused on the functional and morphological characteristics of the microcirculation in the submucosal layer, where another neural plexus is located that plays a critical role in regulating mucosal blood supply (Hirst, 1977; Kotecha & Neild, 1995).

Role of L-type VDCCs. In marked contrast to spontaneous Ca^{2+} transients in mural cells of the venules in the bladder or the submucosa of stomach (Hashitani *et al.* 2011, 2012; Mitsui & Hashitani, 2015*a*), blockade of LVDCCs does not disrupt the generation or synchrony of Ca^{2+} transients in a majority of myenteric capillary pericytes or arteriolar SMCs (Hashitani *et al.* 2015). LVDCCs also play a minimal role in establishing the basal Ca^{2+} concentration of capillary pericytes, (cf. mural cells in the proximal retinal microvasculature; Ishizaki *et al.* 2009). Thus, the relative contribution of LVDCCs to Ca^{2+} transients in the microvasculature appears to vary amongst vascular beds and also exhibit regional differences even within a microvascular network (see Burdyga & Borysova, 2014).

Role of T-type VDCCs. Synchronous mural cell Ca^{2+} transients in the myenteric microvasculature are abolished by blockers of TVDCCs, indicating that these spontaneous Ca^{2+} transients predominately rely on TVDCCs (Fig. 1). TVDCCs are low threshold channels that open at membrane potentials as negative as −70 mV (Perez-Reyes, 2003). The window current for TVDCCs usually occurs over membrane potentials which are more hyperpolarised than that of LVDCCs. (e.g. T-type, −65 mV to −45 mV cf. L-type, −30 mV to 0 mV; Hirano *et al.* 1989). Since the resting membrane potential of retinal or renal pericytes ranged between −60 mV and −50 mV (Sakagami *et al.* 1999; Zhang *et al.* 2001), window currents for TVDCCs in pericytes may well be contribute to these cells exhibiting pacemaker function.

Differences in the contribution of L- and TVDCCs to spontaneous activity are evident in various vascular beds or regions within a microvasculature tree. TVDCCs are functionally expressed in arterial and arteriolar SMCs and contribute to vascular tone (Hansen *et al.* 2001; Jensen *et al.* 2004; Kuo *et al.* 2010, Abd El-Rahman *et al.* 2013). In cerebral arteries, nifedipine-resistant, mibefradil-sensitive vascular tone increases with decreasing vessel size, suggesting that TVDCCs play a larger role in smaller arteries (Kuo *et al.* 2010). Furthermore, the percentage

of TVDCC currents in freshly isolated vascular SMCs increases dramatically along the lower branches of the mesenteric artery, rising to almost 100% in submucosal arterioles (Morita *et al.* 1999). Conducted vasoconstriction in rat mesenteric arterioles <40 μm *in vivo* also relies exclusively on TVDCCs (Gustafsson *et al.* 2001).

Role of SR Ca^{2+} release. While the generation of synchronous Ca^{2+} transients in the myenteric microvasculature relies on TVDCCs, Ca^{2+} transients are also readily abolished by CPA, caffeine or tetracaine, suggesting that both $InsP_3$- and ryanodine-receptors are involved (Parker & Ivorra, 1991). SR store Ca^{2+} release may be the primary event in generating spontaneous Ca^{2+} transients in capillary pericytes, since the inhibition of SERCA with CPA abolishes asynchronous Ca^{2+} transients of pericytes when L- and TVDCCs are previously blocked (Fig. 2). Extracellular Ca^{2+} influx via a pathway other than L- and TVDCCS is also required to maintain the cytosolic Ca^{2+} oscillator in these pericytes, as both synchronous and asynchronous Ca^{2+} transients are abolished by nominally Ca^{2+}-free solution.

A common mechanism underlying pericyte pacemaking and synchronisation is coupled oscillators arising from the reciprocal interaction between Ca^{2+} release from SR and membrane depolarisations (Fig. 1). This has been proposed in lymphatics (Imtiaz *et al.* 2007), arteries and arterioles (Peng *et al.* 2001; Haddock & Hill, 2005) and in the distal gastric antrum (van Helden & Imtiaz, 2003). SR Ca^{2+} release can cause depolarisation by opening CaCCs or cation channels. Pericytes in the descending vasa recta and retina have CaCCs associated with cyclic oscillations in $[Ca^{2+}]_i$ (Sakagami *et al.* 1999; Zhang *et al.* 2008). ICC in the GI tract are known to express TMEM16A/Ano1 CaCCs (Gomez-Pinilla *et al.* 2009; Hwang *et al.* 2009). In the myenteric microvasculature of the stomach, TMEM16A/Ano1 immunofluorescence is detected in myenteric ICC (ICC-MY) but not in the microvasculature. In the mouse small intestine, pericytes associated with the microvasculature near the myenteric plexus express cation permeable, maxi-anion channels and are not immunoreactive for Ano1 (Parsons *et al.* 2012). Thus the Ca^{2+}-activated inward currents that trigger the activation of TVDCCs in myenteric pericytes remain to be established.

Coupled oscillator mechanisms require membrane depolarisation paired with SR Ca^{2+} release (Fig. 1). In pericytes of suburothelial venules of the bladders or venular SMCs in the stomach submucosa, this appears to be mediated by depolarisation associated with the opening of LVDCCs that cause Ca^{2+} influx to activate SR Ca^{2+} release through CICR. In contrast, TVDCCs undertake this role in myenteric pericytes or arteriolar SMCs. Functional coupling between TVDCCs and SR Ca^{2+} release to drive pacemaker mechanisms has been reported in sinoatrial pacemaker cells (Háuser *et al.* 2000) or ICC in the GI tract (Kito & Suzuki, 2003; Zheng *et al.* 2014). Interestingly, in interstitial cells of the guinea-pig prostate, TVDCCs but not LVDCCs stimulate CaCCs, suggesting that TVDCCs are functionally coupled with SR Ca^{2+} release (Lang *et al.* 2014).

Pericytes act as pacemaker cells. Synchronous spontaneous Ca^{2+} transients in the myenteric microvasculature are initiated in the capillaries where the pericytes are distributed. Imaging the cerebral microvasculature *in vivo* shows that capillary dilatation in response to the excitation of vasodilatory neurons precedes arteriolar dilatation, suggesting that hyperpolarising signals initiated in capillaries may spread to arterioles (Hall *et al.* 2014).

Capillary Ca^{2+} transients sometimes fail to spread into neighbouring arterioles, suggesting that there may be gating functions at the junctions between capillaries and arterioles. In addition, active capillaries that initiate spontaneous Ca^{2+} transients do not generally maintain their dominance, often switching randomly from one capillary to another with time. Consistently, studies on the retinal microvasculature indicate that the axial electrotonic voltage transmission is highly efficient, particularly in capillaries, but significant voltage dissipation occurs at branching points (Zhang *et al.* 2011). Although specialised units of SMCs located at arterial branching points may serve as originators of rhythmic vasomotion in the microvasculature of kidney and skin (Colantuoni *et al.* 1985; Goligorsky *et al.* 1995), this is not the case in the myenteric microvasculature tree. Thus, it is reasonable to suggest that capillary pericytes may act as pacemaker cells generating spontaneous Ca^{2+} transients and associated depolarising signals that entrain arterioles to develop highly synchronous Ca^{2+} transients in mural cells of the microvasculature.

Comparison of spontaneous Ca^{2+} transients in ICC-MY with those in neighbouring microvessel indicate there are no temporal correlations (Hashitani *et al.* 2015). Thus, Ca^{2+} transients in capillary pericytes or arteriolar SMCs occur independently of ICC-MY activity. Furthermore, pericytes or SMCs of the myenteric microvasculature and perivascular interstitial cells are not immunoreactive for antibodies against Kit receptor, the universal marker for ICC. Therefore, neither ICC nor Kit-positive interstitial cells found in other visceral organs act as pacemakers driving the spontaneous Ca^{2+} transients in the viseceral microvasculature.

Spontaneous Ca^{2+} transients in the myenteric microvasculature are not affected by tetrodotoxin or electrical field stimulation (EFS). Consistently, PGP9.5-positive perivascular nerve fibres are not found along the

myenteric microvasculature, whereas perivascular nerve fibres containing many varicosities, the sites of neurotransmission, are adjacent to the intramuscular microvasculature. This is in contrast to the microvasculature in the submucosal layer of the GI tract where both arterioles and venules are functionally innervated by sympathetic nerves (Hirst, 1977; Mitsui *et al.* 2013; Mitsui & Hashitani, 2015*a*). Recent 3-D imaging of the myenteric layer of mouse small intestine with a technique termed 'vessel painting' has revealed that the periganglionic capillary network is in close contact with ganglionic cells, as well as glial cells (Fu *et al.* 2013). Nevertheless, spontaneous Ca^{2+} transients in the myenteric microvasculature occur independently from neural activity.

Outlook and concluding remarks

Vasomotion in arterioles has been considered to reduce vascular resistance and enhance blood flow to facilitate tissue oxygenation (Nilsson & Aalkjaer, 2003). Similarly, active venular constrictions produce an increase in the blood flow and exhibit temporal vessel diameter-blood velocity and pressure relationships characteristic of a peristaltic pump (Dongaonkar *et al.* 2012). Although it was believed that valves are absent in fine veins or venules, microvalves in venules that restrict flow from postcapillary venules back into the capillary bed have been demonstrated (Caggiati *et al.* 2006). The presence of valves further enhances the efficiency of the venular peristaltic pump by preventing retrograde flow. Since capillary filtration and reabsorption is a function of hydrostatic pressure that is determined by the ratio of post-to-precapillary resistance, regulation of contractility of 'upstream' arterioles and 'downstream' venules by capillary pericytes seems to be ideal mechanism to adjust microcirculation to meet tissue demands.

In the bladder suburothelium, capillary pericytes express NG2 but not α-SMA, questioning their ability to contract. More recently, NG2-DeRed mice allowed us to visualise spontaneous Ca^{2+} transients in capillary pericytes, but the Ca^{2+} transients are apparently not associated with a reduction in capillary diameter, suggesting that these capillary pericytes are non-contractile (H Hashitani, unpublished data). Similarly, capillary pericytes in myenteric microcirculation of the stomach appear to act as 'non-contractile' pacemaker cells to drive 'upstream' arterioles. In contrast, pericytes in retinal and cerebral capillaries contract upon electrical stimulation or application of excitatory neurotransmitters (Peppiatt *et al.* 2006; Fernández-Klett *et al.* 2010; Hall *et al.* 2014). Such differences in the role of pericytes may reflect characteristics of different microcirculatory beds. Since both the retinal and cerebral microvasculature are located within organs that are relatively inflexible in terms of their architecture and limited volumes, fine regulation and/or distribution of blood supply may be required. On the other hands, more dynamic rearrangements of microvasculature architecture as well as blood flow distribution may well be possible in distensible visceral organs. Therefore, pericytes may play different roles in different microcirculatory beds to meet the characteristics of individual organs.

Hyperpolarising signals initiated in capillary pericytes may spread to arterioles in retinal and cerebral microcirculation and result in the closure of LVDCCs and therefore vasodilatation (Puro, 2012; Hall *et al.* 2014). In contrast, pericyets in visceral organs appear to generate periodical transient depolarisations to drive spontaneous rhythmic vasoconstrictions in 'upstream' arterioles or 'downstream' venules. During progressive distension of the wall in visceral organs that are usually associated with only small rises in transmural pressure, suppressions of contractility of microvessels as seen in the retina and cerebral microcirculation would result in compression or collapse of microvasculature, particularly in venules where the intraluminal pressure is the lowest. Thus, periodical vasomotions rather than simple vasodilatation may be required to maintain microcirculation blood flow. Distension of visceral organs may facilitate pericyte-driven vasomotion by opening stretch-activated channels.

Dysfunction of 'pacemaking' mechanisms of capillary pericytes and/or transmission of 'pacemaking' depolarisations to precapillary arterioles would result in insufficient tissue oxygenation due to the increased resistance against blood flow into capillaries. Disturbances in the spread of 'pacemaking' depolarisations to postcapillary venules would diminish the pumping functions of venules, resulting in stagnation of tissue excreta.

In the normal bladder intravesical pressure rises are minimal, so that it is likely that blood flow of arteries or arterioles is well maintained. However, even small rises in the intravesical pressure may readily attenuate the spontaneous venular constrictions, resulting in the disturbance of suburothelial microcirculation. In addition, the multipotency of pericytes may contribute to the remodelling of the bladder suburothelium that is commonly seen in an overactive bladder. Increased pericyte numbers have been reported in pulmonary microvessels in patients with pulmonary hypertension and a murine retinal angiogenesis model (Ricard *et al.* 2014). Thus, increased pericyte expression may alter contractility of microvasculature and simply reduce substance exchange across capillary walls by reducing the capillary surface area.

Ischaemic and reperfusion damage to the cells in the bladder wall has been proposed as a major cause of overactive bladder (Gosling *et al.* 2000) which accompanies ageing and atherosclerosis (Azadzoi *et al.* 1999; Yoshida *et al.* 2010). The beneficial effects of α-adrenoceptor antagonists on bladder storage symptoms may be attributed to an improvement in bladder microcirculation.

Similarly, β3-adrenoceptor agonists and PDE5 inhibitors may also exert their action by improving the bladder microcirculation. Since these pharmacological agents improve bladder storage function without improving the narrowed luminal diameter of the feeding arteries (Andersson *et al.* 2014), their therapeutic effects may also be attributed to their actions on the contractile and/or morphological properties of microvasculature within the bladder wall.

Further understanding of microvasculature functions in visceral organs, particularly the role of pericytes and VDCCs in the generation of 'spreading' spontaneous activity, may highlight novel therapeutic strategies for functional disorders in visceral organs.

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Additional information

Competing interests

The authors state no conflict of interest.

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