Synchrony of spontaneous Ca\(^{2+}\) activity in microvascular mural cells

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Abstract

Spontaneous rhythmic constrictions known as vasomotion are developed in several microvascular beds \textit{in vivo}. Vasomotion in arterioles is considered to facilitate blood flow, while venular vasomotion would facilitate tissue metabolite drainage. Mechanisms underlying vasomotion periodically generate synchronous Ca\(^{2+}\) transients in vascular smooth muscle cells (VSMCs). In visceral organs, mural cells (pericytes and VSMCs) in arterioles, capillaries and venules exhibit synchronous spontaneous Ca\(^{2+}\) transients. Since sympathetic regulation is rather limited in the intra-organ microvessels, spontaneous activity of mural cells may play an essential role in maintaining tissue perfusion. Synchronous spontaneous Ca\(^{2+}\) transients in pre-capillary arterioles (PCAs)/capillaries appear to propagate to upstream arterioles to drive their vasomotion, while venules develop their own synchronous Ca\(^{2+}\) transients and associated vasomotion. Spontaneous Ca\(^{2+}\) transients of mural cells primarily arise from IP\(_3\) and/or ryanodine receptor-mediated Ca\(^{2+}\) release from sarcoplasmic reticulum (SR/ER) Ca\(^{2+}\) stores. The resultant opening of Ca\(^{2+}\)-activated Cl\(^{-}\) channels (CaCCs) causes a membrane depolarisation that triggers Ca\(^{2+}\) influx via T-type and/or L-type voltage-dependent Ca\(^{2+}\) channels (VDCCs). Mural cells are electrically coupled with each other via gap junctions, and thus allow the sequential spread of CaCC or VDCC-dependent depolarisations to develop the synchrony of Ca\(^{2+}\) transients within their network. Importantly, the synchrony of spontaneous Ca\(^{2+}\) transients also requires a certain range of the resting membrane potential that is maintained by the opening of K\(_V\)7 voltage-dependent K\(^{+}\) (K\(_V\)7) and inward rectifier K\(^{+}\) (K\(_{ir}\)) channels. Thus, a depolarised membrane would evoke asynchronous, ‘premature’ spontaneous Ca\(^{2+}\) transients, while a hyperpolarised membrane prevents any spontaneous activity.

Key words: smooth muscle, pericyte, vasomotion, microvasculature, intracellular calcium

Abbreviations: CaCC: Ca\(^{2+}\)-activated Cl\(^{-}\) channel; CICR: Ca\(^{2+}\)-induced Ca\(^{2+}\) release; IK channel: intermediate-conductance Ca\(^{2+}\)-activated K\(^{+}\) channel; K\(_{ir}\) channel: inward rectifier K\(^{+}\) channel; K\(_V\)7 channel: K\(_V\)7 voltage-dependent K\(^{+}\) channel; LVDC: L-type voltage-dependent Ca\(^{2+}\) channel; NO: nitric oxide; NOS: nitric oxide synthase; PCA: precapillary arteriole; PCV: postcapillary venule; SK channel: small-conductance Ca\(^{2+}\)-activated K\(^{+}\) channel; SMC: smooth muscle cell; SR/ER: sarcoplasmic reticulum; STD: spontaneous transient depolarisation; TVDCC: T-type voltage-dependent Ca\(^{2+}\) channel; VDCC: voltage-dependent Ca\(^{2+}\) channel; VSMC: vascular smooth muscle cell.

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Spontaneous Vasomotion of Microvessels

Arterioles and venules in several vascular beds in vivo exhibit periodic spontaneous constrictions known as spontaneous vasomotion (1). Arteriolar vasomotion is considered to facilitate arteriolar blood flow into capillaries (2–4), while venular vasomotion facilitates venular drainage (5). Since spontaneous vasomotion is also generated in vitro (6), it is likely that the spontaneous activity originates from microvasculature themselves rather than driven by systemic humoral or neuronal factors.

The mural cells, i.e. vascular smooth muscle cells (VSMCs) or pericytes, in the microvasculature of visceral organs exhibit synchronous spontaneous Ca$^{2+}$ transients with or without vasoconstrictions (7, 8). Therefore, visualisations of the spontaneous changes in intracellular Ca$^{2+}$ dynamics of mural cells are fundamental to explore the origin of spontaneous vasomotion in microvascular networks. This short review summarises recent advances in understanding the mechanisms underlying spontaneous Ca$^{2+}$ transients in mural cells, particularly focusing on their synchrony.

Morphological Properties of Microvascular Mural Cells

In capillaries, pericytes with a morphology distinct from spindle-shaped VSMCs have been recognised since the 19th century using various staining methods including silver impregnation (9). Transmission electron microscopy revealed that the basement membrane is not observed between the pericyte and endothelial cell. Thus, pericytes and the endothelium make frequent membranous contacts in capillaries and postcapillary venules (PCVs) (10). Scanning electron microscopy using enzymatically-digested specimens demonstrates that capillary pericytes have an oval cell body with primary processes extending in the longitudinal directions (9, 11, 12). The mural cells of precapillary arterioles (PCAs) have an oval or round cell body with several circumferentially-oriented processes, while PCV mural cells have an oval or round cell body and several processes extending in various directions (9, 11, 12).

In thin whole mount preparations, immunohistochemistry using specific markers for mural cells such as α-smooth muscle actin (α-SMA) or NG2 chondroitin sulphate proteoglycan (NG2) revealed the arrangement of mural cells in different segments of microvessels (13–18). The entire network of microvessels or just the microvascular segment in a single plane can also be visualised by immunohistochemistry using endothelial markers such as endothelial nitric oxide synthase (eNOS, Fig. 1A, B) (16), von Willebrand factor (vWF) (16) or CD31 (19).

Mural cells express immunoreactivity for α-SMA in most microvascular segments with the exception of capillary pericytes in some tissues (13, 15). Consistent with the expression of α-SMA, α-SMA-positive mural cells in arterioles or PCAs of the mouse bladder suburothelium are contractile, while capillary pericytes do not contract during their synchronous spontaneous Ca$^{2+}$ transients (19). In contrast, capillary pericytes in the central nervous system and heart appear to be contractile (20–22) corresponding with their expression of α-SMA immunoreactivity (22, 23).

Morphological characteristics of mural cells in PCVs or venules can also be visualised by immunohistochemistry for α-SMA. At a higher magnification, the stellate-shaped mural cells in PCVs of rat gastric submucosa (Fig. 1C–E) are clearly distinct from the circumferentially-oriented, tightly-packed smooth muscle cells (SMCs) in venules of the same vascular network (Fig. 1F) (16). The heterogeneity of mural cell morphology in different vascular segments of gastric microvasculature is very similar to that in the mouse and rat bladder (15) and also corresponds to scanning electron microscopy observations in the rat mammary gland (11), suggesting
that morphological features of mural cells are preserved in different tissues.

The mural cells of arterioles and capillaries but not venules are immunoreactive for NG2 chondroitin sulphate proteoglycan (NG2) in hollow visceral organs (Fig. 2A–C) (15, 17, 22, 24). Similar expression patterns of NG2 have also been reported in the mesentery (14), subcutaneous tissue (14), skeletal muscles (14) and retina (23). Tg(Cspg4-DsRed.T1)1Akik/J mice (NG2 DsRed mice) have the advantage of allowing the visualisation of
Visualisation of NG2 chondroitin sulphate proteoglycan (NG2)-positive mural cells in precapillary arterioles (PCAs).

Double immunostaining for NG2 (green) and α-smooth muscle actin (α-SMA, red) combined with nuclear staining (blue) reveals the round cell bodies of mural cells (arrows) in the PCAs of rat rectal submucosa (A–C). Smooth muscle cells in the connecting larger arteriole (asterisks) are also faintly immunopositive for NG2. A bladder mucosal specimen of NG2 DsRed mouse demonstrates the NG2-expressing suburothelial microvasculature consisting of a branching arteriolar tree (a), PCAs (pca) and a capillary meshwork (cap) (D). Mural cells in the PCA shows an oval or round cell body and circumferentially-oriented processes (E). Micrographs in A–C are reproduced from (17), and those in D and E are from (19) with permission.
NG2-expressing cells and their morphology (25) in different segments of the microvascular (Fig. 2D, E) (19). In combination with green fluorescent Ca^{2+} indicators, intracellular Ca^{2+} dynamics of the mural cells can be examined in a microvascular segment-specific manner (19). More recently, NG2cre:GCaMP3 mice have allowed intracellular Ca^{2+} imaging of the microvascular mural cells in the somatosensory cortex \textit{in vivo} where rhythmic spontaneous Ca^{2+} transients are generated in the mural cells (26). Interestingly, arterioles and PCAs but not capillaries show detectable spontaneous changes in vessel diameter (26).

**Fig. 3.** Proposed mechanisms underlying synchronous spontaneous Ca^{2+} transients in mural cells. A: Spontaneous Ca^{2+} release from sarco-endoplasmic reticulum (SR/ER) via IP_3 receptor (IP_3R) and/or ryanodine receptors (RyR) triggers the opening of Ca^{2+}-activated Cl^- channels (CaCCs) to depolarise the membrane (ΔV) (cf. reference 41). The CaCC-dependent depolarisation further activates voltage-dependent Ca^{2+} channels (VDCCs). Ca^{2+} influx through VDCCs stimulates Ca^{2+}-induced Ca^{2+} release (CICR) via RyR and/or IP_3R, and CaCC-dependent membrane depolarisation (ΔV) would increase IP_3 production to facilitate IP_3-induced Ca^{2+} release. The sequestration of cytosolic Ca^{2+} is mediated by sarco-endoplasmic reticulum Ca^{2+}-ATPase (SERCA). B: In a rat bladder suburothelial venule, individual spontaneous action potentials of venular smooth muscle cells (upper trace) precede each vasoconstriction as shown by a reduction in venular diameter (lower trace). Traces in B are reproduced from (38) with permission.

**Basis of Spontaneous Ca^{2+} Transients in Mural Cells**

Spontaneous Ca^{2+} transients in mural cells and associated vasomotion are primarily arise from the spontaneous release of Ca^{2+} from the sarcoendoplasmic reticulum (SR/ER) (1, 6–8) as shown in Fig. 3A. Inhibition of SR/ER Ca^{2+}-ATPase with cyclopiazonic acid or thapsigargin abolishes spontaneous Ca^{2+} transients and/or...
vasomotion in arterioles (27), PCAs (28), venules (29) or PCVs (16). Spontaneous Ca\(^{2+}\) or contractile activity is also prevented upon the inhibition of IP\(_3\) and/or ryanodine receptors. In addition, spontaneous Ca\(^{2+}\) cycling is terminated by the blockade of store-operated Ca\(^{2+}\) entry but not the sodium calcium exchanger 3 (24).

Mural cells generate spontaneous transient depolarisations (STDs) that can sum to develop larger ‘pacemaker’ depolarisations to drive spontaneous vasomotion of the microvessels (Fig. 3B). Ca\(^{2+}\) released from SR/ER triggers the opening of Ca\(^{2+}\)-activated Cl\(^-\) channel (CaCC) allowing Cl\(^-\) efflux to depolarise the membrane. The CaCC-dependent depolarisations further activate voltage-dependent Ca\(^{2+}\) channels (VDCCs), resulting in Ca\(^{2+}\) influx that triggers Ca\(^{2+}\)-induced Ca\(^{2+}\) release (CICR) from the SR/ER via ryanodine receptors (Fig. 3A). In addition, the CaCC-dependent depolarisations may facilitate IP\(_3\) production that in turn triggers SR/ER Ca\(^{2+}\) release resulting in the further amplification of CaCC-dependent depolarisations (Fig. 3A) (1, 8). Thus, there seems to be a reciprocal facilitation between SR/ER Ca\(^{2+}\) cycling and plasmalemmal ion channels (1, 8).

STDs arising from the activation of ion channels also play a critical role in maintaining the synchrony of Ca\(^{2+}\) transients amongst mural cells, in turn, vasomotion (as will be discussed later).

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**Roles of Gap Junctions in the Synchrony of Spontaneous Ca\(^{2+}\) Transients**

In the submucosal PCAs of rat rectum, mural cells develop synchronous spontaneous Ca\(^{2+}\) transients (Fig. 4A, B) (17). Similarly, mural cells in gastric PCVs exhibit synchronous spontaneous Ca\(^{2+}\) transients (Fig. 4C) (16) and corresponding spontaneous vasomotion. Carbenoxolone (3 μM), a gap junction blocker, reversibly disrupts the synchrony of Ca\(^{2+}\) transients leaving asynchronous Ca\(^{2+}\) transients in the individual mural cells (Fig. 4C, D) and also attenuates spontaneous vasomotion. In the mouse bladder suburothelium, stellate-shaped PCV pericytes also develop synchronous spontaneous Ca\(^{2+}\) transients and associated vasomotion (19). Carbenoxolone (10 μM) abolishes the spontaneous Ca\(^{2+}\) transients in the mural cells of PCVs or disrupts their synchrony, while preventing Ca\(^{2+}\) transients in venules (19). Thus, the synchrony of spontaneous Ca\(^{2+}\) transients among PCV mural cells depends on intercellular coupling via gap junctions, and such coupling is required for generating spontaneous vasomotion.

Gap junction-mediated intercellular communication between mural cells has been well demonstrated by simultaneous patch clamp recordings of two mural cells in isolated PCAs of the rat kidney (30). Because of spread of the angiotensin II-induced depolarisation in a PCA mural cell to an adjacent endothelial cell, gap junction-mediated communication between mural cell and endothelial cells are indicated (30).

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**Origin of Spreading Synchronous Spontaneous Ca\(^{2+}\) Transients in the Microvasculature**

In the bladder suburothelium of NG2-DsRed mice, ‘non-contractile’ capillary pericytes exhibit synchronous spontaneous Ca\(^{2+}\) transients that propagate to PCAs resulting in diameter changes (19). Carbenoxolone (10 μM) disrupts the synchrony of spontaneous Ca\(^{2+}\) transients in capillary pericytes, while preventing spontaneous Ca\(^{2+}\) transients in mural cells of connecting PCAs, indicating that capillary pericytes function as pacemaker cells to drive the upstream PCAs (8, 19). In the guinea-pig stomach, synchronous spontaneous Ca\(^{2+}\) transients in PCA mural cells spread to SMCs of arterioles to evoke spontaneous vasomotion (28).

Thus, in arteriole-capillary networks, synchronous spontaneous Ca\(^{2+}\) transients in mural cells appear to be predominantly generated in PCAs or capillaries and can retrogradely propagate to arterioles to evoke
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Fig. 4. Roles of gap junction in maintaining the synchrony of spontaneous Ca$^{2+}$ transients. In a submucosal precapillary arteriole (PCA) of rat rectum loaded with Cal-520, mural cells with a round shaped cell body exhibit spontaneous Ca$^{2+}$ transients (A). Four mural cells are randomly selected as regions of interests (ROIs). Corresponding traces demonstrate the synchrony of spontaneous Ca$^{2+}$ transients in the four cells (B). In a postcapillary venule (PCV) of rat gastric submucosa loaded with Cal-520, spontaneous Ca$^{2+}$ transients in five mural cells are synchronous (C). Carbenoxolone (3 μM), a gap junction blocker, disrupts the synchrony of spontaneous Ca$^{2+}$ transients amongst the five cells with a reduction in their amplitude. The gradual disruption of the synchrony is evident in the merged traces corresponding to C (D). Images and/or traces are reproduced from (17) (A, B) and (16) (C, D) with permission.
spontaneous vasomotion. The spreading nature of synchronous spontaneous Ca\(^{2+}\) transients may explain the fact that spontaneous vasomotion is preferentially observed in small arteriolar branches less than 20 μm in diameter \textit{in vitro} (18, 19, 27) and \textit{in vivo} (2, 26, 31–34).

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**Spontaneous Depolarisations as a Means of the Synchrony of Spontaneous Ca\(^{2+}\) Transients**

\textit{In vitro} studies have demonstrated rhythmically generated pacemaker potentials arising from summated STDs in VSMCs of the rat irideal or basilar arterioles (27, 35) or human pial arteries (36). Pacemaker potentials are associated with Ca\(^{2+}\) transients and corresponding spontaneous vasoconstrictions. Rhythmic pacemaker depolarisations in venular SMCs of the cat gastric submucosa are also associated with spontaneous constrictions (37).

In the lamina propria preparation of rat bladder, pacemaker potentials of venular SMCs precede each spontaneous vasoconstriction (Fig. 3B) (38). The resting membrane potential of spontaneously-active venular SMCs in the rat and mouse bladder suburothelium is about −43 mV and −45 mV, respectively (19, 38). These values are close to the activation threshold of L-type voltage-dependent Ca\(^{2+}\) channels (LVDCCs) (39). Indeed, blockade of LVDCCs suppressed slow waves and disrupted their synchrony amongst venular SMCs leaving asynchronous STDs, indicating that STDs sum to trigger the opening of LVDCCs to generate slow waves and associated vasomotion (19). The spontaneous vasomotion is associated with synchronous spontaneous Ca\(^{2+}\) transients in circumferentially-oriented SMCs or stellate pericytes in bladder venules (24), supporting the notion that synchronous Ca\(^{2+}\) influx through LVDCCs in these cells is required for the generation of spontaneous vasomotion.

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**Roles of Voltage-dependent Ca\(^{2+}\) Channels in the Synchrony of Spontaneous Ca\(^{2+}\) Transients**

Inhibitors of LVDCCs, nifedipine or nicardipine, disrupt the synchrony of spontaneous Ca\(^{2+}\) transients in the mural cells of venules (Fig. 5A) and inhibit spontaneous venular vasomotion (16, 24, 29, 38, 40). Thus, the intercellular coupling amongst venular mural cells appears to be mediated by the spread of LVDCC-dependent depolarisations, presumably via gap junctions. Nifedipine also disrupts the synchrony of spontaneous Ca\(^{2+}\) transients in the SMCs of basilar arterioles and abolishes their vasomotion (35).

In contrast to venules or arterioles, the synchrony of spontaneous Ca\(^{2+}\) transients in PCA mural cells of the guinea-pig gastric myenteric layer (28), rat rectal submucosa (17) and mouse bladder suburothelium are not disrupted by the blockade of LVDCCs (Fig. 5B). In contrast, the blockade of T-type voltage-dependent Ca\(^{2+}\) channels (TVDCCs) by ML218 or mibebradil disrupts the synchrony of spontaneous Ca\(^{2+}\) transients among mural cells in the PCAs of guinea-pig stomach (28). However, blockade of TVDCCs decreases their frequency in PCAs of rat rectum without disrupting their synchrony (Fig. 5C) (17). In the rat rectal PCA mural cells, TVDCCs appears to be involved in regulating the frequency of spontaneous Ca\(^{2+}\) transients, while LVDCCs contributes to the duration of spontaneous Ca\(^{2+}\) transients (Fig. 5B, C) (17).
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Roles of Ca\(^{2+}\)-activated Cl\(^{-}\) Channels in Generating Synchronous Spontaneous Ca\(^{2+}\) Transients

The synchrony of spontaneous Ca\(^{2+}\) transients among mural cells in the rat rectal PCAs is not affected by blockers of LVDCCs and TVDCCs but disrupted by lowering extracellular Cl\(^{-}\) from 134.4 mM to 12.4 mM or in the presence of a Ca\(^{2+}\)-activated Cl\(^{-}\) channel (CaCC) blocker (17). Lowering extracellular Cl\(^{-}\) or CaCC blocker also disrupts the synchrony of spontaneous Ca\(^{2+}\) transients among mural cells and/or inhibits spontaneous vasomotion in several vascular beds (16, 35, 38). Thus, spontaneous Ca\(^{2+}\) release from SR/ER opens CaCCs to cause membrane depolarisations that appear to play a fundamental role in intercellular signal spread. The inhibitors of Na\(^{+}\)-K\(^{+}\)-Cl\(^{-}\) co-transporter bumetanide and furosemide decrease the amplitude of spontaneous Ca\(^{2+}\) transients and resultant constrictions without disrupting their synchrony (16), suggesting that Cl\(^{-}\) accumulation in mural cells is partly dependent on these co-transporters and that other Cl\(^{-}\) accumulation mechanisms such as anion exchangers may also be operating (41).

![Fig. 5](image-url)

Fig. 5. Roles of voltage-dependent Ca\(^{2+}\) channels (VDCCs) in maintaining the synchrony of spontaneous Ca\(^{2+}\) transients.
In a submucosal venule of rat rectum, nifedipine (1 μM), an L-type VDCC blocker, disrupts the synchrony of spontaneous Ca\(^{2+}\) transients among four regions of interest (A). In a submucosal precapillary arteriole (PCA) of rat rectum, two mural cells exhibit synchronous spontaneous Ca\(^{2+}\) transients, and nifedipine does not affect the synchrony of spontaneous Ca\(^{2+}\) transients, while their duration is reduced by nifedipine (B). Mibebradil (1 μM), a T-type VDCC blocker, also has no effect on the synchrony of spontaneous Ca\(^{2+}\) transients of two PCA mural cells in the rat rectum (C). Mibebradil decreases their frequency. Traces are reproduced from (17) with permission.
CaCC-dependent depolarisations are also fundamental in generating slow waves in interstitial cells of Cajal (ICC), gastrointestinal pacemaker cells, to electrically drive SMCs (42, 43) or rhythmic spontaneous constrictions of lymphatic vessels (44–46). Consistent with this, immunoreactivity for TMEM16A (also known as ANO1), a CaCC, is detected in ICC (47) or lymphatic SMCs (46). In contrast, TMEM16A immunoreactivity is not detected in mural cells of the microvascular (24, 29), suggesting that the mural cells may have other types of CaCCs such as TMEM16B (48).

Roles of K+ channels in maintaining the synchrony of spontaneous Ca2+ transients

In the PCA mural cells of the rat rectum, the Kv7 voltage-dependent K+ (Kv7) channel blocker XE 991 (10 μM, Fig. 6A) or an increase in [K+]o from 5.9 mM to 29.7 mM converts synchronous Ca2+ transients into asynchronous, high-frequency Ca2+ transients (49). Thus, Kv7 channels in PCA mural cells of the rectum appear to be constitutively open to maintain their relatively hyperpolarised membrane potential to prevent ‘premature’ asynchronous Ca2+ transients in individual cells. Since subsequent application of nifedipine decreases their frequency but fails to recover their intercellular synchrony (Fig. 6A), the activation of LVDCCs appears to be involved in the generation of asynchronous, high-frequency Ca2+ transients, but not a critical cause of the disruption of intercellular synchrony. Levromakalim, a KATP channel opener that is known to hyperpolarise mural cells (19), restores the synchrony of spontaneous Ca2+ transients (Fig. 6B), suggesting that repolarisations of the mural cell membrane potential are required to restore the synchrony.

Ba2+ (50 μM), a known blocker of inward rectifier K+ (Kir) channels also changes the synchronous spontaneous Ca2+ transients into asynchronous, high-frequency Ca2+ transients (Fig. 7A) (49). Thus, some Kir channels in the endothelial cells and/or mural cells of rectum PCAs appear to be open under resting condition to prevent asynchronous, high-frequency Ca2+ transients. A small increase in [K+]o from 5.9 mM to 10.7 mM, which is known to activate Kir channels (50), inhibits the spontaneous Ca2+ transients, presumably by opening more Kir channels, this hyperpolarising the membrane of mural cells (50). Consistent with the functional Kir channel (Kir2.1) expression in PCAs, immunoreactivity for Kir2.1 is detected in the endothelial cells but not mural cells in the submucosal PCAs in the rat rectum (Fig. 7B–G) (49) as is the case of endothelial cells of brain capillaries (51). These data indicate that Kir channel-dependent hyperpolarisations generated in the endothelium can be transmitted to mural cells via myoendothelial gap junctions. Nevertheless, other subtypes of Ba2+-sensitive Kir channel subunits may also be expressed in the mural cells or the endothelium. Functional expression of Kir channels has been demonstrated in PCA mural cells of the rat kidney (vasa recta) and retina (52, 53).

The blockade of both small (SK)- and intermediate (IK)-conductance Ca2+-activated K+ channels has no effect on the synchronous spontaneous Ca2+ transients in the PCA mural cells of rat rectum (49). Consistently, endothelial cells of mouse brain capillaries do not express SK and IK channels (51), suggesting that capillaries or connecting PCAs do not have functional SK/IK channels. This is in contrast to SMCs of rat mesenteric arteries, in which endothelial SK and IK channel play a role in maintaining the intercellular synchrony of phenylephrine-evoked rhythmic Ca2+ transients and resultant vasomotion (54, 55). Large conductance Ca2+-activated K+ channels are not involved in the synchronisation of spontaneous Ca2+ transients in the rat basilar artery (35) and rectal PCA (49), while these channels contribute to suppress the basal Ca2+ level in the basilar artery. Thus, Ca2+-activated K+ channels only play a marginal role in maintaining the synchrony of spontaneous Ca2+ transients in the PCA mural cells.

A hyperpolarised membrane potential suppresses VDCC-mediated Ca2+ influx and resultant CICR as well
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as IP$_3$ receptor-mediated Ca$^{2+}$ release from the SR/ER (Fig. 8A). In the conditions where ‘premature’ Ca$^{2+}$ transients are prevented, SR/ER Ca$^{2+}$ stores are allowed to be fully refilled so that regenerative Ca$^{2+}$ release is periodically generated. Regenerative Ca$^{2+}$ releases trigger CaCC-dependent depolarisations that are sufficiently large in their amplitude to spread to distant cells. Since levcromakalim alone suppresses or abolishes spontaneous Ca$^{2+}$ transients (49), setting the resting membrane potential within a certain range appears to be critical for generating synchronous Ca$^{2+}$ transients (Fig. 8B).

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Fig. 6. Roles of K$_v$7 voltage-dependent K$^+$ (Kv7) channels in maintaining the synchrony of spontaneous Ca$^{2+}$ transients. In three mural cells of rat rectal precapillary arteriole (PCA), XE 991 (10 μM), a blocker of K$_v$7 channels, converts synchronous spontaneous Ca$^{2+}$ transients into asynchronous, high-frequency Ca$^{2+}$ transients and increases the basal Ca$^{2+}$ level (A). Subsequent nifedipine decreases their frequency but does not restore the synchrony. In an XE991-treated rectal PCA where asynchronous spontaneous Ca$^{2+}$ transients are generated, levcromakalim, an ATP-sensitive K$^+$ channel opener that is known to hyperpolarise mural cells, restores their intercellular synchrony (B). Traces are reproduced from (49) with permission.
Roles of Endothelium in Maintaining the Synchrony of Ca$^{2+}$ Transients

Nitric oxide (NO) released from the endothelium appears to play a critical role in maintaining the synchrony of spontaneous activity of mural cells in the rat gastric PCVs. Tadalafil, an inhibitor of phosphodiesterase type 5 (PDE5), disrupts the synchrony of Ca$^{2+}$ transients in the mural cells and inhibits associated spontaneous vasomotion of gastric PCVs (16). Since tadalafil failed to inhibit vasomotion in PCVs that had been pre-treated with nitric oxide synthase (NOS) inhibitor, constitutive PDE5 activity in the mural cells counteracts NO/cGMP signalling to maintain the synchronous Ca$^{2+}$ transients.

In arterioles, the endothelial layer functions as a low-resistance pathway that allows the conduction of electrical signals from adjacent SMCs via myoendothelial gap junctions to distant SMCs (56). Such electrical...
Fig. 8. Fundamental roles of the membrane potential in maintaining the synchrony of spontaneous Ca\(^{2+}\) transients. A: K\(_{V7}\) voltage-dependent K\(^{+}\) (K\(_{V7}\)) channels and inward rectifier K\(^{+}\) (K\(_{ir}\)) channels are open under resting condition to hyperpolarise the mural cells. This hyperpolarisation inhibits voltage-dependent IP\(_3\) production and subsequent IP\(_3\) receptor (IP\(_3\)R)‐mediated Ca\(^{2+}\) release and also decreases voltage-dependent Ca\(^{2+}\) channel (VDCC)‐mediated Ca\(^{2+}\) influx and subsequent Ca\(^{2+}\)‐induced Ca\(^{2+}\) release via ryanodine receptors (RyR) and/or IP\(_3\)R. A resultant decrease in the frequency of spontaneous Ca\(^{2+}\) release from the sarcoplasmic reticulum (SR/ER) ensures the enough Ca\(^{2+}\) refilling in the SR/ER; thus, each spontaneous Ca\(^{2+}\) release from SR/ER is large enough to induce Ca\(^{2+}\)‐activated Cl\(^{−}\) channel (CaCC)‐mediated depolarisation that leads to recruitment/activation of more store Ca\(^{2+}\) release events and their synchronisation within the network of mural cells. B: When the resting membrane potential of mural cells are within the ‘synchronous range’, cyclical spontaneous Ca\(^{2+}\) release from SR/ER (blue line) opens CaCCs to induce cyclical spontaneous depolarisation (black line). The depolarisation causes L-type voltage-dependent Ca\(^{2+}\) channel (LVDCC)‐mediated Ca\(^{2+}\) influx (red line). These spontaneous activities spread to neighbouring mural cells via gap junctions. When the resting membrane potential of mural cells are within the ‘quiescent range’, voltage‐dependent IP\(_3\) production is suppressed. Thus, cyclical spontaneous Ca\(^{2+}\) release from SR/ER is now not generated (blue flat line), and membrane potential change is not detected (black flat line). When the resting membrane potential is higher than the threshold of LVDCC, i.e., within the ‘asynchronous range’, both cyclical spontaneous Ca\(^{2+}\) release from SR/ER (blue line) and LVDCC‐mediated Ca\(^{2+}\) influx (red line) cause high frequency spontaneous Ca\(^{2+}\) transients that are generated independently among mural cells.
coupling has also been demonstrated between mural cell and endothelial cells in PCAs or capillaries (30, 51). Thus, it is envisaged that the synchrony of spontaneous Ca\textsuperscript{2+} transients in mural cells in the PCA or capillary depends on the low-resistance endothelial layer rather than electrical coupling between mural cells. Indeed, in brain capillaries where the bipolar pericytes are sparsely distributed, the tips of the elongated processes of two adjacent pericytes come within a very close proximity, but do not contact or overlap, indicating a lack of a direct electrical coupling between pericytes (57).

**Putative Physiological roles of Microvascular Synchronous Activity**

Increases in the intraluminal pressure of the dog gastric corpus (58) and colon (59) induce wall distention resulting in the reduction of mucosal and/or submucosal blood supply (i.e., ischaemia) associated with a diminished oxygen consumption in the mucosa (59). Spontaneous vasomotion in submucosal arterioles and venules is expected to facilitate the mucosal circulation and thus may well preserve oxygen/nutrients supply to the mucosa even during organ wall distensions.

Synchronous Ca\textsuperscript{2+} transients in the mural cells of capillaries or PCAs propagate to upstream arteriolar branches to evoke vasomotion in the mouse bladder (19) and guinea-pig stomach (28). Since capillaries are the site of substance exchange between blood and tissue where metabolic demand and environmental changes (e.g., oxygen, nutrients and pH) can be finely sensed, it is reasonable that synchronous spontaneous Ca\textsuperscript{2+} transients originate in capillary pericytes and spread to upstream PCAs or arterioles to drive spontaneous vasomotion (7, 49). This will meet the tissue demands of oxygen/nutrients and reset the tissue pH appropriately.

Sympathetic nerve projections become sparser along PCAs and PCVs in the mouse bladder suburothelium (19) and virtually absent in capillaries of the mouse bladder suburothelium and myenteric plexus of mouse stomach (19, 60). Thus, these microvascular segments appear not to be under tight sympathetic control, suggesting that their spontaneous activity plays a critical role in maintaining their perfusion. This is in contrast to PCAs/capillaries in the central nervous system, where the blood flow appears to be precisely regulated by neural activity (20, 26) as well as by their own spontaneous activity (26).

The disruption of spontaneous activity of microvascular mural cells may underlies disease states, and thus could a therapeutic target. Diabetic rat models show an impairment of contractility of mural cells in the retinal PCA/capillary network (61). Gap junctions-mediated intercellular coupling of mural cells is also disrupted in the PCA/capillary of diabetic rat retina (61). Therefore, further investigations into changes in spontaneous arteriolar vasomotion (4) in the diabetic human eye are clinically relevant. Ischaemia in the rat heart or brain causes the sustained contraction and eventual rigor mortis of mural cells in capillaries and/or PCAs, suggesting their critical roles in developing no-flow phenomenon after reperfusion of upstream arteries (21, 22). In visceral organs, the causal relationship between circularity dysfunction of the bladder and lower urinary tract symptoms, particularly overactive bladder, is well documented. Since previous studies focused on bladder ischaemia subsequent to the occlusion of bladder feeding arteries (62), microvasculature within the bladder wall that regulates the transmural distribution of blood supply should be a fruitful research target. More specifically, functions and dysfunctions of the suburothelial microvascular network that play a key role in determining the mucosal blood flow in the bladder storage phase should be explored.
Conclusions

Besides the previously established mechanisms underlying spontaneous Ca\(^{2+}\) transients in mural cells, namely the reciprocal interaction between SR/ER Ca\(^{2+}\) cycling and plasmalemmal ion channels, the significance of the resting membrane potential in maintaining the synchrony of spontaneous Ca\(^{2+}\) transients has become increasingly evident. While the spread of regenerative depolarisations triggered by Ca\(^{2+}\) transients is critical for the intercellular coupling, K\(^+\) channels also play a fundamental role in maintaining the resting membrane potential within an appropriate range. Future studies investigating changes in the resting membrane potential in disease states and means of its restoration will be of great interest.

Conflict of Interest

The authors declare no conflict of interest.

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