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Ryanodine receptors, calcium signaling and regulation of vascular tone in the cerebral parenchymal microcirculation

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Abstract

The cerebral blood supply is delivered by a surface network of pial arteries and arterioles from which arise (parenchymal) arterioles that penetrate into the cortex and terminate in a rich capillary bed. The critical regulation of cerebral blood flow, locally and globally, requires precise vasomotor regulation of the intracerebral microvasculature. This vascular region is anatomically unique as illustrated by the presence of astrocytic processes that envelope almost the entire basolateral surface of parenchymal arterioles. There are, moreover, notable functional differences between pial arteries and parenchymal arterioles. For example, in pial vascular smooth muscle cells (VSMCs), local calcium release events ("calcium sparks") through ryanodine receptor (RyR) channels in sarcoplasmic reticulum membrane activate large conductance, calcium-sensitive potassium (BK) channels to modulate vascular diameter. In contrast, VSMCs in parenchymal arterioles express functional RyR and BK channels, but under physiological conditions these channels do not oppose pressure-induced vasoconstriction. Here we summarize the roles of ryanodine receptors in the parenchymal microvasculature under physiologic and pathologic conditions, and discuss their importance in the control of cerebral blood flow.

Keywords

brain parenchymal arteriole; cerebral blood flow; acidosis; ryanodine receptor; potassium channel

Introduction

In vascular smooth muscle cells (VSMCs), a global increase in cytoplasmic calcium concentration ($[Ca^{2+}]_i$) leads to vasoconstriction by activation of Ca^{2+}/calmodulin-dependent myosin light-chain kinase. However Ca^{2+} is a versatile second messenger, and vasomotor pathways involving local Ca^{2+} signals, such as Ca^{2+} sparks encoded by ryanodine receptors (RyRs), or propagated Ca^{2+} signals in the form of Ca^{2+} waves, have been the subject of many recent studies. The topic of Ca^{2+} signaling in the microcirculation is one of growing interest, particularly in the cerebral parenchyma as brain integrity is critically dependent on constantly adapting blood perfusion. Arteriolar tone significantly depends on the Ca^{2+} entry through ion channels of the VSMC plasma membrane, and also on the Ca^{2+}-dependent vasodilator influences of endothelial cells. However these aspects of microvascular Ca^{2+} signaling are beyond the scope of the current review. The goal of this review is to provide a synopsis of current knowledge regarding RyR-dependent Ca^{2+} signals

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in smooth muscle cells of the brain parenchymal microvasculature and how these signals influence cerebral blood flow (CBF).

Part 1: Regulation of cerebral blood flow by parenchymal arterioles

Within the brain microcirculation, the parenchymal arterioles (PAs) are fundamental regulators of CBF. PAs account for approximately 40% of the total cerebral vascular resistance (33). Thus regulation of their diameter is a central process for (i) maintaining constant perfusion of brain tissue across a range of systemic arterial pressures (cerebrovascular autoregulation), and (ii) during neurovascular coupling (NVC), *i.e.* the mechanisms by which functional hyperemia occurs in the brain (34, 58).

1.1 Myogenic tone and cerebrovascular autoregulation

A function of arteries and arterioles is to constrict and relax in response to changes in intravascular pressure. An increase in intraluminal pressure constricts cerebral arteries and arterioles. A decrease in intravascular pressure has the opposite effect, inducing vasodilation. This essential regulatory mechanism, known as the vascular myogenic response was first described more than one hundred years ago (9) and ensures that blood flow remains nearly constant during moment-to-moment fluctuations in arterial pressure. PAs are the last smooth muscle-containing vessels upstream of the capillary bed and play a critical role in maintaining appropriate capillary perfusion pressure under both normal and pathologic conditions (33, 55). Additional anatomical features of the brain microcirculation, particularly the limited collateral supply blood flow, which has a so-called “bottleneck” effect on perfusion of the neocortex (87), further emphasize the relative importance of this part of the intracerebral microcirculation and its control mechanisms including those involved in regulation of myogenic tone.

The precise nature of the mechanisms and modulators of myogenic vasoconstriction is still a matter of some debate. However, myogenic constriction occurs in blood vessels studied in isolation, demonstrating that mechanisms intrinsic to the vascular wall are sufficient to induce this response (90, 119). Disruption of the endothelium does not impair pressure-induced constriction suggesting that both sensor and effector mechanisms responsible for the myogenic response reside within smooth muscle cells (75). In turn, it is broadly accepted that the pressure-induced constriction involves a depolarization of the arterial myocyte cell membrane (52) that activates voltage-dependent Ca^{2+} channels (VDCC), resulting in Ca^{2+} influx and subsequent vasoconstriction (62). Interestingly, compared with pial arteries, PAs depolarize and constrict to lower levels of intravascular pressure leading to a high amount of tone (between 30% and 40% at 40 mm Hg) (23, 29, 32, 51, 86, 88). Physiologically, this creates a high “vasodilator reserve”, i.e. a substantial capacity of these arterioles to dilate in response to locally generated vasodilator signals. This is particularly adapted to CBF regulation and the functional hyperemic response, as the vascular resistance is inversely proportional to the fourth power of the arterial radius according to the Hagen-Poiseuille equation.

1.2 The neurovascular unit regulates CBF locally

The ability of the brain to coordinate neural activation state with local CBF has been recognized since the end of the nineteenth century (102). This linkage is called neurovascular coupling. It underlies functional hyperemia in the brain, which ensures that local increases in metabolic demand are satisfied by increased substrate delivery by the blood. Impairment of this coupling between neuronal metabolism and cerebral perfusion, even if brief, generally trigger dramatic consequences. Anatomic and functional observations support a role of capillaries in functional hyperemia (59, 94). However, PAs

are thought to mediate a large part of the local CBF increase in response to increased neuronal activity, due to their high level of vascular resistance and ability to dilate rapidly in response to a variety of endogenous substances (34, 37, 44, 110, 133). In contrast to pial arteries, PAs are not supplied with perivascular nerves (49) but are wrapped in astrocytic processes, called endfeet, which cover nearly their entire basolateral surface (58, 106). Studies over the last ten years have illuminated the importance of this anatomical configuration in mediating NVC. Synaptic release of neurotransmitters such as glutamate during brain neuronal activity stimulates metabotropic receptors on astrocytes and generates an increase in cytoplasmic Ca^{2+} which propagates through astrocytic processes by activation of inositol 1,4,5-trisphosphate receptors ($InsP_3Rs$), ultimately elevating $[Ca^{2+}]_i$ in the endfeet. There, this Ca^{2+} signal activates pathways leading to dilation of the PAs, involving various factors such as locally released K^+ ions, adenosine, prostaglandins or epoxyeicosatrienoic acids (EETs) (37, 58, 96, 108). Neurons also release several substances able to regulate CBF such as nitric oxide (NO), prostanoids and vasoactive neurotransmitters (5, 57). Therefore neurons, astrocytes, myocytes and endothelial cells form a neurovascular unit that collectively controls local blood flow. These various pathways leading to vasodilation all act, to some extent, by hyperpolarizing the VSMC membrane which deactivates VDCC and then decreases $[Ca^{2+}]_i$.

Thus, it is clear that the intracerebral microcirculation participates actively and substantially in the global and local regulation of CBF. The control of parenchymal arteriolar diameter by the VSMCs within the vessel wall is a process that is fundamental to this regulation. In turn, regulation of VSMC tone depends on variations in cytoplasmic $[Ca^{2+}]_i$. Therefore, understanding the regulation of $[Ca^{2+}]_i$ in smooth muscle cells of PAs is of central importance.

Part 2: Roles of the sarcoplasmic reticulum, inositol 1,4,5-trisphosphate receptors and ryanodine receptors in regulation of parenchymal arterioles

2.1 The sarcoplasmic reticulum and regulation of intracellular Ca^{2+}

The sarcoplasmic reticulum (SR) is constantly involved, through the release and re-uptake of Ca^{2+} into the cytoplasm, in vascular smooth muscle Ca^{2+} homeostasis (123). At low intraluminal pressure (e.g. 10 mm Hg), VSMC $[Ca^{2+}]_i$ has been estimated to be ~120 nM (62, 88). The sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) transports Ca^{2+} from the cytoplasm into the SR. The presence in the SR lumen of the Ca^{2+} binding proteins, calreticulin and calsequestrin (65), also facilitates the active pumping of Ca^{2+} against its gradient by decreasing the luminal Ca^{2+} concentration (123). As a result, the available Ca^{2+} in the SR is significant, and therefore the opening of SR Ca^{2+} channels leads to the release of Ca^{2+} into the cytosol (54, 97).

Various smooth muscle cellular functions, including contraction, relaxation, proliferation and differentiation, involve Ca^{2+} release from the SR (13, 54, 123). Two families of SR Ca^{2+} release channels have been identified so far: the $InsP_3R$ and the RyR.

2.2 Inositol 1,4,5-trisphosphate receptors

The $InsP_3R$ consists of 4 subunits with a molecular weight of ~310 kDa each (40). Three distinct genes code three different types of subunits leading to three receptor subtypes ($InsP_3R1$-3). Although all three isoforms appear to be expressed in vascular myocytes, $InsP_3R1$ seems to be the predominant subtype in adult VSMCs, and $InsP_3R2$ and $InsP_3R3$ appear to be expressed primarily during proliferative phases of cellular activity (69, 79, 83, 112, 129). The possible formation of heterotetrameric complexes has been demonstrated by co-immunoprecipitation of $InsP_3R$ expressed in cell lines (77).

All $InsP_3R$ subtypes are activated by inositol 1,4,5-trisphosphate ($InsP_3$) (38), which is produced by phospholipase C (PLC) activation in response to $G\alpha_{q/11}$ protein coupled receptor activation (11, 13, 40). Therefore, $InsP_3Rs$ are involved in the responses to several endogenous vasoactive molecules such as endothelin 1, acetylcholine, noradrenaline, and 5-HT (12). $InsP_3Rs$ also display a bell shaped Ca^{2+} sensitivity: $InsP_3R$-Ca^{2+} release is potentiated at low Ca^{2+} concentrations (< 500 nM) whereas it is inhibited at higher Ca^{2+} concentrations (> 1~10 μM), with differences between subtypes (15, 38, 40, 115). These properties appear to be fundamental in the generation of Ca^{2+} oscillations observed in VSMCs in response to vasoconstrictors (12). Recently, in pial arteries, $InsP_3R$-mediated Ca^{2+} release has been shown to activate transient receptor potential TRPM4 channels (46) and large conductance Ca^{2+}-activated potassium (BK) channels (130). Activation of transient receptor potential TRPC3 channels by direct physical coupling with $InsP_3R$ has also been described (124).

2.3 Ryanodine receptors

Similarly to $InsP_3Rs$, three different genes encode three different RyR subtypes (RyR1-3) and functional channels are formed by the association of four subunits (35, 50). Possible heteromeric RyR2/RyR3 and RyR2/RyR1 formations, but not RyR1/RyR3 formations, have been demonstrated by immunoprecipitation (127). To date, the RyR is the only ion channel discovered by electron microscopy (41), presumably due to the strikingly large size of RyR protein subunits (550–660 KDa) (89). RyR subunits have cytosolic C- and N-termini and between four and twelve transmembrane domains, depending on the four different models proposed (19, 111, 117, 134). The reason(s) for the well-conserved large size of the RyRs, and the associated substantial metabolic cost of production and maintenance, remain unclear but underscores the physiological importance of these proteins.

RyR1 and RyR2 have been extensively studied in skeletal muscle and cardiac muscle, respectively, (66) where they are highly expressed and play a fundamental role in excitation-contraction (E-C) coupling. RyR3 appears to be more broadly expressed, with predominance in neurons (43, 68). The three subtypes are expressed in large cerebral arteries (118) as well as in the cerebral microcirculation (Dabertrand & Brayden, unpublished observations). Their expression in VSMCs also appears to be modulated by pathologic and environmental conditions (25, 26, 30, 65, 78). The channel opening is regulated by several agents including SR Ca^{2+} load (35, 89). But RyRs seem regulated primarily by binding of Ca^{2+} on the cytosolic face of the channel. $[Ca^{2+}]_i$ ranging from 1 to 10 μM triggers the opening of the channel, whereas millimolar $[Ca^{2+}]_i$ inhibits it (35, 60). Consequently, RyRs can be activated by Ca^{2+} entry through plasma membrane Ca^{2+} channels or by SR Ca^{2+} release from adjacent $InsP_3Rs$ or RyRs (13). This mechanism, called Ca^{2+}-induced Ca^{2+} release (CICR), is fundamental in cardiac contraction (66, 98). CICR has been described in several types of smooth muscle including cerebral arteries (61), in which bolus application of caffeine, which simultaneously activates all the RyRs, causes transient increases in $[Ca^{2+}]_i$ and vasoconstriction in both pial and parenchymal arterioles (29, 63). However, in contrast to cardiac and skeletal myocytes, global RyR-Ca^{2+} release appears to be minimal during E-C coupling in VSMCs under physiological conditions (60, 120).

In contrast to the excitatory role of RyRs in skeletal and cardiac muscle, RyRs mediate local Ca^{2+}-release events (Ca^{2+} sparks) that activate BK channels to oppose pressure-induced depolarization and contraction of cerebrovascular smooth muscle (60, 63, 85, 120). The serial linkage of RyR and BK in this negative feedback vasodilator mechanism is well established, and clearly demonstrated by the robust and non-cumulative constrictions induced by RyR and BK channel blockers in intact pressurized pial arteries (63, 64). Interestingly, small caliber pial arterioles and PAs do not constrict to BK channel blockers *in vivo* (91, 109) or *in vitro* (29, 44, 51, 56), despite functional BK channels in VSMCs of

these arterioles (29, 51). This suggests that under basal conditions the functional role of RyRs in the cerebral microcirculation is distinct from pial arteries.

Part 3: Calcium signaling in parenchymal arteriolar myocytes

3.1 Waves rather than sparks in the microcirculation

Under normal conditions of pH and temperature, pressure-induced constriction of intracerebral parenchymal arterioles is poorly counterbalanced by Ca^{2+} spark-driven BK current (29, 51, 56). This absence of negative feedback likely contributes to the greater myogenic tone observed in PAs compared to pial arteries (23, 24, 88). Using the intracellular fluorescent Ca^{2+} dye Fluo-4, we found that VSMCs in pressurized PAs exhibited primarily Ca^{2+} waves (78% of cells) propagating through the cytoplasm over a duration of several seconds, and very few localized Ca^{2+} events (12% of cells) (29). Ca^{2+} waves and Ca^{2+} sparks were not observed in PAs when SR Ca^{2+} was depleted by cyclopiazonic acid or when the arterioles were exposed to RyR blockers (ryanodine or tetracaine). These results indicate that RyRs mediate both Ca^{2+} waves and Ca^{2+} sparks in PAs (29). However, it is premature to conclude that waves rely exclusively on RyRs with no $InsP_3R$ involvement (47). Interestingly, a similar Ca^{2+} signal topography, *i.e.* Ca^{2+} sparks in large arteries and Ca^{2+} waves in the microcirculation, has also been described in cremaster muscle feed arteries and arterioles (121, 122). However, Westcott and Jackson (2011) attributed the waves in the arterioles not to RyR, but to $InsP_3R$ activation. Differences in $InsP_3R$ and RyR subtype expression between the cremaster and the cerebral microcirculation may account for this divergence (25, 27, 28, 73, 121). Moreover, the presence of perivascular nerves in cremaster arterioles (39) may facilitate the engagement of the $InsP_3R$ in the generation of Ca^{2+} waves by releasing $G\alpha_{q/11}$ protein coupled receptor agonists, and thus maintaining a high level of cytoplasmic $InsP_3$. Receptor tyrosine kinase activation also generates $InsP_3$ (13) and possible contributions of this signaling pathway should be considered. In contrast to cremaster arterioles, PAs lack extrinsic innervation (49) and demonstrate elevated cytoplasmic $[Ca^{2+}]_i$ (88) which may promote RyR activation. The large variability observed in the mechanisms triggering and propagating Ca^{2+} waves in smooth muscle makes it difficult to develop a global model, but the variation in RyR function between large and small caliber arteries remains noteworthy and requires further detailed investigation.

In a recent study, Mufti et al. provided interesting insights into the role of the RyR-mediated Ca^{2+} waves in cerebral arteries. They demonstrated their importance in maintaining myogenic tone under low pressure by increasing phosphorylation of myosin light chain (82). Parenchymal arterioles depolarize and constrict to a greater extent than pial arteries at lower pressure (23, 51, 88). These observations are consistent with (i) the predominance of Ca^{2+} waves in PA VSMCs and (ii) the lack of effect of RyR and BK channel blockers on PA diameter. A likely explanation for the inability of Ca^{2+} waves to activate BK channels is that Ca^{2+} waves do not deliver sufficient Ca^{2+} to activate BK channels (95). In contrast, Ca^{2+} sparks deliver micromolar Ca^{2+} to closely apposed BK channels to cause substantial channel activation.

3.2 Local Ca^{2+} release initiates Ca^{2+} waves

The ability of Ca^{2+} sparks to initiate Ca^{2+} waves which travel through the cytoplasm following a CICR mechanism was first described in cardiac myocytes (20, 21) and is still an area of active investigation (18). Similarly, Ca^{2+} spark-initiated Ca^{2+} waves have been observed in several vascular beds including the hepatic portal vein (4, 48), small mesenteric arteries (99), and retinal arterioles (116). The prevalent model explaining the propagation of the initial Ca^{2+} release to the entire cell is that a local increase in Ca^{2+} activates neighboring

RyRs because resting open state probability (P_o) of RyRs is high (Figure 1) (10, 13, 16). When resting P_o is low, local RyR Ca^{2+} release (e.g. a spark) is not sufficient to activate nearby RyRs to cause a Ca^{2+} wave (Figure 1) (10, 13). It is also possible that ambient $InsP_3$ levels are elevated in PAs, and that a combination of $InsP_3$Rs and RyRs in non-junctional SR elements is causing Ca^{2+} waves (47).

The fundamental role played by the RyR P_o in shaping intracellular Ca^{2+} signals is particularly well illustrated in the observations reported by Heppner et al. in VSMCs of pial arteries (53). These investigators found that a small increase in pH (7.4 to 7.5) increases Ca^{2+} spark frequency. However, further increases in pH beyond 7.6, shifted Ca^{2+} signaling from stationary sparks to Ca^{2+} waves (Figure 1) (53). The simplest explanation for this phenomenon is that a decrease in intracellular proton (H^+) levels increased RyR P_o, and therefore the likelihood of Ca^{2+} waves. However, the induced Ca^{2+} waves contributed little to the observed vasoconstriction (53).

3.3 Acidosis converts Ca^{2+} waves to sparks to activate BK channels

Because smooth muscle cells in PAs exhibit mostly Ca^{2+} waves at physiological pH, we hypothesized that acidic pH might dilate PAs by reshaping the predominant intracellular Ca^{2+} signal from Ca^{2+} waves to Ca^{2+} sparks, which would activate BK channels to cause membrane potential hyperpolarization and vasodilation (Figure 1). Using pharmacological and genetic approaches, we found that this mechanism accounts for 60% of the vasodilation observed by acidifying the external pH from 7.4 to 7.0 (29). The concept that acidosis, over a narrow and physiologically relevant pH range, can induce vasodilation principally by remodeling Ca^{2+} signals is entirely novel, and as described below, may not be limited to the intracerebral microcirculation.

The reshaping of the Ca^{2+} signaling in VSMCs of PAs by protons likely occurs because changes in extracellular pH (pH_o) lead to changes in intracellular pH (pH_i). The relationship between pH_o and pH_i has been established in VSMCs from many tissues including the brain (3, 6, 92, 113). This relationship appears to be linear with a ratio $\Delta pH_i/\Delta pH_o$ of about 0.73 (7). Moreover, both normocapnic and hypercapnic acidosis induce a RyR-BK channel component of acidic pH-induced dilation. In a 1989 study, Toda et al. investigated the relaxation of dog cerebral artery strips in response to acidosis induced by hypercapnia or manipulation of the bicarbonate concentration (114). These investigators showed that the increase in CO_2 from 5% to 15%, as in our study (29), has a vasorelaxant effect mediated by decreasing pH rather than acting through direct effects of CO_2 in the bathing media. This finding is consistent with our observations concerning the similarity between normocapnic and hypercapnic acidosis effects on Ca^{2+} signaling in PAs. Additionally, a reduction in pH_o lowers cytoplasmic Ca^{2+} (8) which would decrease RyR activity. Interestingly, Heppner et al. showed that inhibition of Ca^{2+} influx through VDCC did not prevent the induction or the persistence of alkaline pH-induced Ca^{2+} waves in rat cerebral pial arteries (53). Thus the reshaping of RyR-dependent Ca^{2+} signaling by pH_o appears to be due primarily to the direct regulation of RyR P_O. The simplest interpretation of the observed remodeling of Ca^{2+} signals at pH 7.0 is that protons lower RyR P_O and then prevent local Ca^{2+} release to activate neighboring RyRs (Figure 1) and Ca^{2+} release remains limited to single RyR (Ca^{2+} quark) or clusters of RyRs (Ca^{2+} sparks). However we have not directly assessed this mechanism and more complex models can be considered. Intracellular acidification in combination with the subsequent reduction of intracellular Ca^{2+} could also decrease the P_o of both RyRs and $InsP_3$Rs, and thereby terminate Ca^{2+} waves. The subsequent reduced leak of Ca^{2+} from the SR would then elevate SR luminal [Ca^{2+}], and thereby may enhance local spark probability at the junctional SR elements which abut the plasma membrane with BK channels (131). Indeed it is well demonstrated and recognized that an increase in the SR Ca^{2+} load activates RyRs (35) and increases Ca^{2+} spark frequency along with spontaneous transient outward

currents occurrence (131). Unfortunately the influence of SR Ca^{2+} load on $InsP_3Rs$ is less clear and experimental evidence is scarce. A luminal Ca^{2+} binding site has been described (104) and Bezprozvanny & Ehrlich showed that $InsP_3R$ activity is inhibited when luminal Ca^{2+} concentration is raised from 3 μM to 10 mM (14), whereas Bottman and coworkers reported a potentiation of the $InsP_3$-induced Ca^{2+} release by luminal Ca^{2+} (17). These issues remain to be resolved and are important topics for future investigation.

3.4 RyRs and neurovascular coupling

Parenchymal arterioles play a critical role during NVC (34). These arterioles dilate in response to neuronal activation, which results in a local increase in blood flow (58, 67, 96). Activation of BK channels by Ca^{2+} sparks through RyR is a powerful vasodilator mechanism and can be activated in PAs by acidification (29). However, the origin and nature of Ca^{2+} signals that occur in parenchymal VSMCs during NVC remain to be identified. Interestingly, in brain slices, VSMCs of PAs exhibit synchronous Ca^{2+} oscillations (36, 37) which are rapidly suppressed during NVC simulated through neuronal depolarization by electric field stimulation. This observation suggests that Ca^{2+} signals in these arterioles are influenced by neural stimulation via vasoactive substances released from astrocytes and neurons. In newborn piglets, glutamate increases Ca^{2+} spark frequency and decreases cytoplasmic Ca^{2+} concentration in the VSMCs of brain slice arterioles (126). This effect is thought to be mediated by carbon monoxide (CO) derived from astrocytic heme oxygenase metabolism (70, 125, 126). However, CO has been described as a tonic vasoconstrictor, preventing H_2S-induced vasodilation in cerebellar slices from neonatal mice (80) and CO failed to dilate isolated pial arteries from adult rats and mice (2). Therefore, the involvement of CO during NVC in the mature brain remains to be established. Others have suggested that epoxyeicosatrienoic acids (EETs) produced by cytochrome P450 epoxygenase may mediate NVC (72, 76, 93, 103). The mode of action of EETs in NVC has not been fully resolved, but these compounds induce BK channel-mediated hyperpolarization of VSMCs (42), and increase Ca^{2+} spark frequency in pial arteries (31). Consequently, EETs are potential modulators of RyR activity during NVC. As in the case of acidosis, Ca^{2+} spark-driven BK currents can be activated in PAs and can induce large, sustained and reversible vasodilation (29) (Figure 1). Then Ca^{2+} spark-driven activation of BK channels may also play a role in the vasodilator effects of substances such as CO and EETs, whereby VSMC relaxation occurs.

In their classic study, Roy and Sherrington suggested a possible role for H^+ during NVC (102). Protons produced by neuronal metabolism could cause local acidification and then vasodilation. While this hypothesis has been challenged, (58, 74) local release of protons from the astrocytic endfoot onto the smooth muscle could produce dilation. Indeed, alkalinization of astrocytic cell body during cortical stimulation (22) or metabotropic glutamate receptor activation (1) has been reported, which would be consistent with the release of H^+. However, using the fluorescent pH indicator SNARFR-5, we did not observe changes in endfoot pH in brain slices following electrical field stimulation (Dabertrand and Nelson, unpublished observations). It is more likely that vasodilation induced by a decrease in pH within the brain may occur during pathologic conditions.

In normal conditions, appropriate pH levels are maintained by the lungs and kidneys. The CO_2 resulting from metabolism is expired by the lungs, but if it is retained or rapidly eliminated, it causes respiratory acidosis or respiratory alkalosis, respectively, The kidney, as a regulatory organ, adjusts acid and base excretion and dysregulation in this balance leads to metabolic acidosis or metabolic alkalosis. Nevertheless, acidification of nervous tissue can be triggered rapidly with reduced respiratory rate, during CO_2 inhalation (132) or during cerebral lactic acidosis related to ischemia (101) and hypoxia (71). Indeed, without oxygen, the brain switches to anaerobic glycolysis, leading to accumulation of lactic acid. Therefore,

during ischemia, tissue pH falls by as much as 1 unit (84, 105) and this acidification is thought to play a role in the injury caused, for example, by ischemic stroke (81, 107, 128). Consequently moderate hyperventilation of patients with cerebral ischemia has been considered in the past to counteract the acidification. However this also increases vascular tone and therefore globally decreases CBF, which worsens the ischemia. (100). Consequently, blockade of downstream targets activated by lactic acidosis appears currently more promising clinically (81). Nevertheless, in the case of cerebral hypoxia or ischemia, acidosis-related vasodilation of PAs might represent a mechanismto maintain perfusion by promoting collateral blood flow.

Conclusion

Since the discovery of Ca^{2+} sparks in smooth muscle in 1995 (85), many roles of RyRs in vascular function have come into focus. Ca^{2+} release through RyRs contributes to Ca^{2+} waves in VSMCs of pressurized arterioles, but the role of Ca^{2+} waves and their contribution to parenchymal arteriolar tone remains obscure. It is also possible that Ca^{2+} waves modulate the activity of Ca^{2+}-dependent transcription factors such as Nuclear factor of activated T-cells (NFAT) (45), and thereby exert transcriptional control. It is, however, clear that at physiological external pH, Ca^{2+} waves do not activate BK channels in VSMCs of pressurized parenchymal arterioles. Acidification leads to a loss of Ca^{2+} waves and the appearance of Ca^{2+} sparks causing profound, rapid and reversible vasodilation. Over a narrow and physiologically relevant pH range, the majority of the proton-induced vasodilation depends on Ca^{2+} spark-driven activation of BK channels (29). These results suggest that the role of the RyRs in vascular smooth muscle is context-dependent. The lack of Ca^{2+} sparks and absence of BK channel-mediated hyperpolarization to oppose pressure-induced constriction at physiological pH can explain why PAs are more depolarized and constricted at lower pressures, an appropriate physiological response for arterioles that experience lower pressures. Acidification-induced Ca^{2+} spark engagement of BK channels provides a novel mechanism to enhance cerebral blood flow, and may play an important role in pathological responses in the brain. Therefore, elucidating the regulatory mechanisms of RyRs is crucial for understanding the control of cerebral blood flow.

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Abbreviations used

$[Ca^{2+}]_i$	intracellular calcium concentration
BK	large conductance Ca^{2+}-activated potassium channel
CBF	cerebral blood flow
CICR	Ca^{2+}-induced Ca^{2+} release
CO	carbon monoxide
CO_2	carbon dioxide

E-C	excitation-contraction
EET	epoxyeicosatrienoic acid
H^+	proton
$InsP_3$	inositol 1,4,5-trisphosphate
$InsP_3R$	inositol 1,4,5-trisphosphate receptor
K^+	potassium
NFAT	nuclear factor of activated T-cells
PA	parenchymal arteriole
pH_i	intracellular pH
pH_o	extracellular pH
P_o	open state probability
RyR	ryanodine receptor
SERCA	sarco/endoplasmic reticulum Ca^{2+}-ATPase
SR	sarcoplasmic reticulum
VDCC	voltage-dependent Ca^{2+} channel
VSMC	vascular smooth muscle cell

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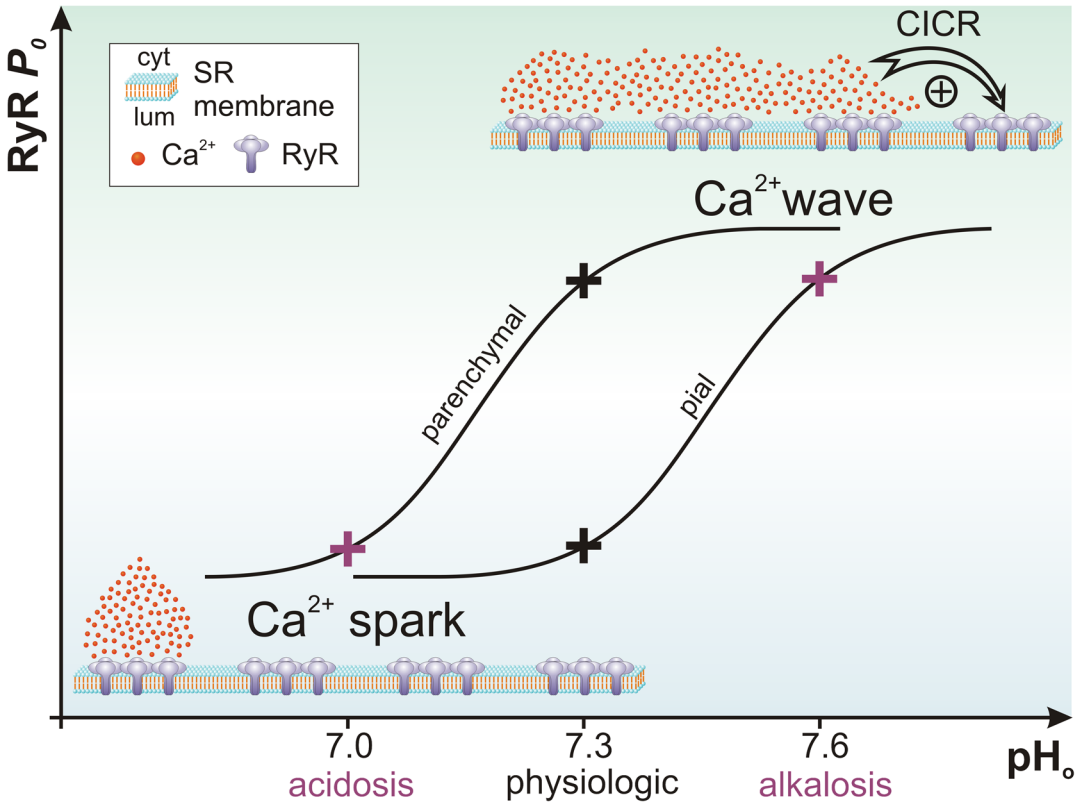

Figure 1. Effect of pH on RyR-mediated Ca^{2+} signals in smooth muscle cells of pressurized pial arteries and parenchymal arterioles

By modulating RyR excitability, protons can influence the shape of the Ca^{2+} signals in cerebrovascular smooth muscle cells. At the physiologic pH of cerebrospinal fluid (pH=7.3) myocytes in large (pial) arteries display spatially limited and brief Ca^{2+} release events (sparks). When pH increases (alkalosis), RyR open probability (P_o) increases as well and the Ca^{2+} ions released by a cluster of RyRs activate neighboring clusters, forming a Ca^{2+} wave by propagation of the signal. In contrast, parenchymal arterioles display Ca^{2+} waves under normal conditions. When pH decreases (acidosis), RyR P_o is low and this spatially restricts the Ca^{2+} release (Ca^{2+} spark). This reshaping of Ca^{2+} signals in the brain microcirculation plays a fundamental role during the vasodilation induced by acidosis.