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Large-conductance Ca^{2+}-activated potassium channels are potently involved in the inverse neurovascular response to spreading depolarization

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ABSTRACT

Recurrent spreading depolarizations occur in the cerebral cortex from minutes up to weeks following acute brain injury. Clinical evidence suggests that the immediate reduction of cerebral blood flow in response to spreading depolarization importantly contributes to lesion progression as the wave propagates over vulnerable tissue zones, characterized by potassium concentration already elevated prior to the passage of spreading depolarization. Here we demonstrate with two-photon microscopy in anesthetized mice that initial vasoconstriction in response to SD triggered experimentally with 1 M KCl is coincident in space and time with the large extracellular accumulation of potassium, as shown with a potassium indicator fluorescent dye. Moreover, pharmacological manipulations in combination with the use of potassium-sensitive microelectrodes suggest that large-conductance Ca^{2+}-activated potassium (BK) channels and L-type voltage-gated calcium channels play significant roles in the marked initial vasoconstriction under elevated baseline potassium. We propose that potassium efflux through BK channels is a central component in the devastating neurovascular effects of spreading depolarizations in tissue at risk.

1. Introduction

Spreading depolarization (SD) is the generic term for all waves of abrupt, near-complete breakdown of the neuronal transmembrane ion gradients that cause cytotoxic edema and propagate at about 3 mm/min in cerebral gray matter. The SD continuum describes the spectrum from short-lasting SDs in metabolically intact tissue to SDs of intermediate duration to terminal SD in severely ischemic tissue (Dreier and Reiffurth, 2015; Hartings et al., 2017). Accordingly, SDs occur in neurological disorders such as the apparently harmless migraine aura, aneurysmal subarachnoid hemorrhage, traumatic brain injury, malignant hemispheric stroke, or circulatory arrest (Dreier et al., 2009; Farkas et al., 2010; Lauritzen et al., 2011; Hinzman et al., 2014; Woitzik et al., 2013; Dreier et al., 2018).

SD is associated with a cerebral blood flow (CBF) response consisting of distinct phases (Ayata and Lauritzen, 2015). The main

Abbreviations: aCSF, artificial cerebrospinal fluid; APG-2, Asante Potassium Green 2 (a K^+-sensitive fluorescent dye); BK channel, large-conductance Ca^{2+}-activated potassium channel; CBF, cerebral blood flow; DC potential, direct current potential; $[K^+]_e$, extracellular K^+ concentration; LFP, local field potential; rSD, recurrent spreading depolarization; SD, spreading depolarization; SD1, the first spreading depolarization in a train of events; VGCC, voltage-gated Ca^{2+} channel; VSMC, vascular smooth muscle cell

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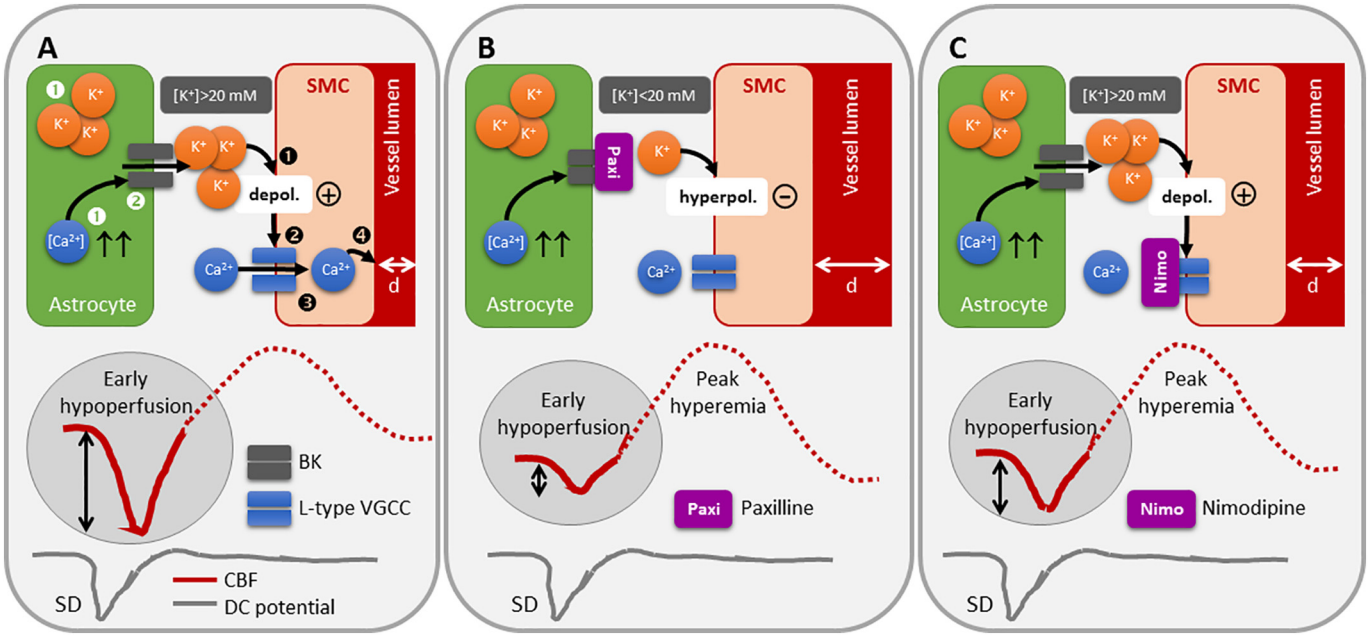

Fig. 1. Hypothesis driving the study, and pharmacological interventions utilized to prove the proposed concept. A, Spreading depolarization (SD; lowermost gray line) causes K^+ accumulation in the interstitium (Somjen, 2001; Pietrobon and Moskowitz, 2014); in turn, astrocytes take up surplus K^+ (Larsen and MacAulay, 2017), and a parallel rise of intracellular Ca^{2+} concentration occurs (Attwell et al., 2010; Li et al., 2012) (white ① in Astrocyte). The increased concentration of Ca^{2+} opens large-conductance Ca^{2+}-activated K^+ (BK) channels at the endfeet to allow K^+ efflux to the periarteriolar space (Girouard et al., 2010) (white ② in Astrocyte). Potassium accumulating at high concentration at the perivascular space (> 20 mM) drives the vascular smooth muscle cell (VSMC) membrane potential towards depolarization (Koide et al., 2012) (black ① in SMC). In turn, VSMC voltage-sensitive Ca^{2+} channels (VGCC) open (balck ② in VSMC) to give way to Ca^{2+} influx (black ③ in VSMC), which induces vasoconstriction (Koide et al., 2012) (black ④ in VSMC). This molecular signaling cascade is believed to underlie the initial hypoperfusion element of the cerebral blood flow (CBF) response to SD. B, Paxilline, a blocker of BK channels, is thought to be selective at the astrocyte endfeet given at concentrations below 1 μM (Girouard et al., 2010), and hinder the periarteriolar accumulation of K^+, thereby promoting hyperpolarization. As a result, VGCCs stay closed, and the vessel lumen is prone to be dilated. Paxilline administration, therefore, is expected to mitigate the early hypoperfusion element of the CBF response to SD. C, Nimodipine, a selective L-type VGCC blocker is applied to target VSMC channels (Alborch et al., 1995), K^+ current from astrocytes being presumably unaltered. As a direct consequence, Ca^{2+} influx is obstructed, vasoconstriction becomes hindered, and the initial hypoperfusion element of the CBF response to SD is anticipated to be smaller in magnitude.

hyperemic element of the CBF response is preceded by a short-lasting hypoperfusion especially discernible in the mouse brain (Fig. 1A). This early hypoperfusion has been identified as the most important parameter in the spectrum from normal hyperemic responses to inverse, vasoconstrictive responses, because it appears to override ensuing hyperemia in the ischemic cortex, thereby jeopardizing the survival of metabolically compromised tissue (Shin et al., 2006; Bere et al., 2014). Because ruling vasoconstriction (i.e. spreading ischemia) hampers the activation of energy-dependent membrane pumps such as the Na^+/K^+-ATPase, hence, the recovery from SD (Major et al., 2017), it prolongs the neuronal depolarization, the cytotoxic edema and the associated toxic intraneuronal Ca^{2+} and Na^+ surge. All these events together augment the risk of irreversible injury. Despite major clinical relevance, the mechanisms behind the CBF reduction in response to SD have not been fully elucidated (Dreier, 2011).

Under physiological conditions, astrocytes mediate vasodilation via Ca^{2+}-dependent activation of large conductance K^+ (BK) channels and K^+ release from astrocytic endfeet (Filosa et al., 2006). In turn, inward rectifier K^+ channels of vascular smooth muscle cells (VSMC) open, the VSMC membrane hyperpolarizes, and voltage-gated L-type Ca^{2+} channels (L-type VGCC) close, as long as the local rise in $[K^+]_e$ remains below 20 mM. Potassium triggers constriction of cerebral vessels at a concentration over 20 mM (Kuschinsky et al., 1972; Golding et al., 2000). SD is associated with a more than ten-fold elevation of $[K^+]_e$ from a baseline level of 3–4 mM to 30–60 mM (Vyskocil et al., 1972; Somjen, 1979), thus reaching the vasoconstrictive concentration range. From the beginning, K^+ has been a prime suspect to be involved in the initial constrictive element of the neurovascular response to SD (Dreier et al., 1998; Windmüller et al., 2005).Transition from astrocyte-evoked vasodilation to vasoconstriction occurs when the intra-astrocytic Ca^{2+} signal approximately doubles from normally 300–400 nM to 700–800 nM at the endfeet (Girouard et al., 2010). In this situation, BK channel activation is strongly enhanced, because the probability of BK channels to be open increases 16-fold when the cytoplasmic Ca^{2+} concentration doubles (Horrigan and Aldrich, 2002). As a consequence, local $[K^+]_e$ in the restricted perivascular space might exceed 20 mM. Under this condition, VSMCs depolarize, because the K^+ equilibrium potential declines, and L-type VGCCs open (Windmüller et al., 2005). Influx of Ca^{2+} then causes VSMCs to contract (Koide et al., 2012) (Fig. 1). Our main goal was to prove the key role of K^+ in the mediation of SD-related vasoconstriction, and to explore the involvement of BK channels and L-type VGCCs (Fig. 1).

It appears from previous investigations that the early vasoconstriction following SD may also be augmented if $[K^+]_e$ prior to or in between SD events is elevated over the physiological range (Dreier et al., 2000). Indeed, in experimental models of focal cerebral ischemia or subarachnoid hemorrhage, baseline $[K^+]_e$ was found to increase from 3 to 6–9 mM (Petzold et al., 2005; Hansen, 1977; Hansen and Zeuthen, 1981). In addition to predisposing the vessels to constrict in response to SD, higher baseline $[K^+]_e$ also predicted longer SD duration in brain slices, unrelated to vascular tone (Dreier et al., 2001). These experimental data have prompted us to analyze how the kinetics of K^+ accumulation with SD may be related to baseline $[K^+]_e$ prior to SD occurrence.

Changes in $[K^+]_e$ have been traditionally acquired with K^+-selective microelectrodes (Vyskocil et al., 1972; Hansen and Zeuthen, 1981), which lack spatiotemporal resolution necessary to establish coupling with CBF accurately. Recently, a novel K^+-sensitive fluorescent dye,