

## Arterial elementary calcium signaling in aging

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Arterial blood pressure increases during aging [1]. Peripheral arteries and arterioles contribute to the increased systemic vascular resistance in hypertension. Resistance sized arteries adapt to hemodynamic forces through changes in their diameter. Myogenic tone is the ability of the vascular smooth muscle cells (VSMC) in the blood vessel wall to constrict when the vessel is radially stretched to cause blood flow autoregulation [2]. A number of artery-related changes contributing to age-dependent increases in blood pressure have been identified: increased vessel stiffness, vascular calcification, alterations by age-associated inflammatory processes, structural changes/vascular remodeling, e.g. increased passive media cross-sectional area [3, 4].

Others and we have focused on mechanisms controlling VSMC contraction/relaxation states to regulate vessel diameter. An indirect mechanism involving elementary  $\text{Ca}^{2+}$  release events ( $\text{Ca}^{2+}$ -sparks) has been identified to limit myogenic tone. This functional unit involves type 2 ryanodine receptors (RyR2s) [2], large-conductance  $\text{Ca}^{2+}$ -sensitive  $\text{K}^+$  ( $\text{BK}_{\text{Ca}}$ ) channels, causing spontaneous transient outward  $\text{K}^+$  currents (STOCs),  $\text{Ca}_v1.2$  L-type  $\text{Ca}^{2+}$  channels, and sarcoplasmic reticulum (SR)  $\text{Ca}^{2+}$ -ATPase (SERCA). Recent data imply a novel role of  $\text{Ca}^{2+}$ -influx via  $\text{Ca}_v3.2$  T-type channels to trigger  $\text{Ca}^{2+}$ -sparks [5, 6], contributing to ~25% of all  $\text{Ca}^{2+}$ -sparks triggered at physiological membrane potentials in mesenteric arteries.  $\text{Ca}_v3.2$  apparently activate RyR2s from the cytosolic side [7], possibly due to the low unitary conductance of T-type channels, therefore the triggering comprises low amounts of total  $\text{Ca}^{2+}$  transferred into the cells and a more restricted spatial range than  $\text{Ca}^{2+}$ -sparks triggered by L-type  $\text{Ca}_v1.2$  channels. Consistent with this mechanism, proper function of the T-type  $\text{Ca}_v3.2$ -RyR axis requires sufficiently high SR  $\text{Ca}^{2+}$  load, which is regulated *via*  $\text{Ca}^{2+}$ -influx through L-type  $\text{Ca}_v1.2$  channels [7].

There is a large body of experimental knowledge on key control processes of VSMC contractile state. However, the studies were usually performed in young adult rodents, whereas hypertension is a typical cardiovascular risk factor in humans at advanced age. We therefore extended our studies to aged mice, using approaches of pharmacological blockade (L-type channels:  $\text{Cd}^{2+}$ , T-type channels:  $\text{Ni}^{2+}$ , TRP channels:  $\text{Gd}^{3+}$ ) and specific knockout mice ( $\text{Ca}_v1.2$ -KO/SMKO,  $\text{Ca}_v3.2$ -KO, EHD2-KO, Caveolin-1-KO).

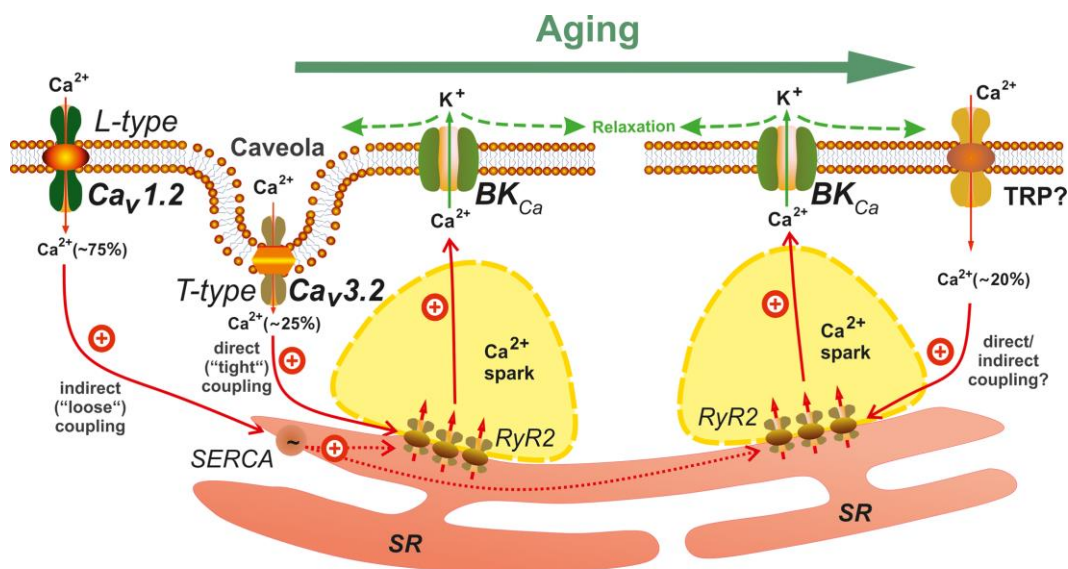
We found that  $\text{Ca}_v1.2$  L-type channels contribute to the same extent to  $\text{Ca}^{2+}$ -sparks generation in young and aged mice. RyR2 is the predominant RyR isoform responsible for  $\text{Ca}^{2+}$ -sparks [3], probably not only for  $\text{Ca}_v1.2$ -mediated  $\text{Ca}^{2+}$ -sparks but also for additional pathways. We found that the  $\text{Ca}_v1.2$ -RyR2 axis works efficiently in old VSMCs, i.e. increasing age does not impair the formation of  $\text{Ca}^{2+}$ -sparks in old VSMCs [7].

Although  $\text{Ca}_v3.2$ -deficiency (KO) does not affect blood pressure in young mice [8],  $\text{Ca}_v3.2$  channels limit myogenic tone in young, but surprisingly not in old mice mesenteric arteries [3, 7, 8]. Decreased  $\text{Ca}_v3.2$  channel expression/activity to trigger  $\text{Ca}^{2+}$  sparks are possible underlying mechanisms for these effects [7]. The newly discovered  $\text{Ca}_v3.2$ -T-type channel  $\text{Ca}^{2+}$ -influx pathway obviously exists only in young mice, but not in mice at advanced age.

Advanced age can alter the composition of lipid rafts and caveolae, which could affect a variety of signaling molecules contributing to the pathophysiology of cardiovascular diseases. Since localization of  $\text{Ca}_v3.2$  in caveolae close to RyRs is essential for triggering  $\text{Ca}^{2+}$ -sparks, we analysed age-related alterations in caveolae to explain the complete malfunction of the  $\text{Ca}_v3.2$ -RyR2 axis in old mice VSMCs. Treatment with methyl- $\beta$ -cyclodextrin, depleting cholesterol as an essential caveolae component from the plasma membrane, failed to inhibit  $\text{Ca}^{2+}$ -sparks in VSMCs of old mice. Electron microscopy images revealed a decrease in caveolae density and alterations in caveolae structure in advanced age [7]. Thus, defective caveolae-RyR2 coupling might be caused by age-related ultrastructural alterations of caveolae and impaired  $\text{Ca}_v3.2$ - $\text{Ca}^{2+}$  sparks signaling by putative caveolemmal T-type  $\text{Ca}_v3.2$  channels, which are insufficiently close to RyRs for extracellular  $\text{Ca}^{2+}$ -influx through T-type channels to trigger  $\text{Ca}^{2+}$ -sparks. Of note, we confirmed our findings using novel Eps15 homology domain-containing protein (EHD2)-deficient mice with genetically impaired caveolae formation. In this animal model,  $\text{Ca}_v3.2$ -expression was also decreased. Together, reduced caveolae density could downregulate  $\text{Ca}_v3.2$  T-type channels to impair the ability of T-type  $\text{Ca}_v3.2$  to generate  $\text{Ca}^{2+}$ -sparks in old VSMCs.

In mice at advanced age, there was a fraction of  $\text{Ca}^{2+}$ -sparks remaining after L-type channel block. This finding enabled us to study effects of the non-specific TRP channel blocker  $\text{Gd}^{3+}$  (100  $\mu\text{M}$ ) to shed a light on the putative role of an TRP channel(s) in VSM elementary  $\text{Ca}^{2+}$  release ( $\text{Ca}^{2+}$ -sparks) to compensate for loss of  $\text{Ca}_v3.2$  T-type channels. We found that caveolae are presumably not required for the gadolinium-sensitive  $\text{Ca}^{2+}$ -influx pathway, since caveolae disruption by methyl- $\beta$ -cyclodextrin did not alter  $\text{Ca}^{2+}$ -spark events in old VSMCs. Due to the low specificity of  $\text{Gd}^{3+}$ , further work is required to ascertain which TRP cation channel(s) or pathways are responsible for generation of these  $\text{Ca}^{2+}$  sparks.

Our results revealed age-dependent differences in elementary  $\text{Ca}^{2+}$  signaling and myogenic tone regulation. The  $\text{Ca}_v3.2$ -RyR-axis is an effective pathway to generate  $\text{Ca}^{2+}$ -sparks in young VSMCs, but plays little or no role in aged VSMCs. A  $\text{Gd}^{3+}$ -sensitive  $\text{Ca}^{2+}$ -influx pathway, putatively through nonselective TRP channels, compensates for loss of the  $\text{Ca}_v3.2$ -RyR-axis. Clarifying the exact identity of the receptor(s)/pathways and the nature of tight vs. loose coupling between  $\text{Ca}^{2+}$  influx-RyR2s pathways (including possible further participating proteins, e. g. SERCA, IP3Rs) will reveal potential novel targets for antihypertensive therapies in patients in the elderly (Figure 1).



**Figure 1. Main  $\text{Ca}^{2+}$  influx pathways regulating  $\text{Ca}^{2+}$ -sparks in young and aged mesenteric vascular smooth muscle cells (VSMCs).** Opening of clustered RyRs in the SR produces  $\text{Ca}^{2+}$ -sparks that activate  $\text{BK}_{\text{Ca}}$  channels providing a negative feedback effect on vasoconstriction.  $\text{Ca}_v1.2$  L-type channels contribute to global cytosolic  $[\text{Ca}^{2+}]$ , thereby influencing luminal SR calcium (via SERCA) and generating the majority (75%) of  $\text{Ca}^{2+}$ -sparks. Caveolae position  $\text{Ca}_v3.2$  T-type channels close to RyRs for extracellular  $\text{Ca}^{2+}$ -influx to trigger (~25%)  $\text{Ca}^{2+}$ -sparks. In aged mice VSMCs, this  $\text{Ca}_v3.2$ -RyR pathway is lost. Instead, a gadolinium-sensitive  $\text{Ca}^{2+}$ -influx pathway triggering (20%)  $\text{Ca}^{2+}$ -sparks is upregulated. Nonselective TRP channels might be involved in this pathway.  $\text{BK}_{\text{Ca}}$ ,  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  channels; RyR2, ryanodine receptor subtype 2; SERCA, sarcoplasmic/endoplasmic calcium pump; SR, sarcoplasmic reticulum.

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