Orchestrating Ca^{2+} influx through Ca$_V$1.2 and Ca$_V$3.x channels in human cerebral arteries

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Vascular tone, and thus blood flow to a tissue bed, is regulated by neuronal-, endothelial-, and smooth muscle–dependent mechanisms. The smooth muscle–dependent component, reflecting the increase in arterial tone in response to increasing pressure (the myogenic response), has been appreciated for over 100 yr (Bayliss, 1902). This myogenic response is an important component of cerebral blood flow (CBF) autoregulation, in which cerebral arteries constrict to an increase in blood pressure so as to maintain constant CBF. Elevation of intraluminal pressure leads to smooth muscle membrane depolarization and ultimately activation of voltage-dependent Ca^{2+} (Ca$_V$) channels. This results in the influx of Ca^{2+} into the cytosol and the initiation of smooth muscle contraction. Dihydropyridine-sensitive, L-type Ca$_V$ channels, in particular Ca$_V$1.2, figure prominently in the development of myogenic tone (Rubart et al., 1996; Knot and Nelson, 1998). Recently, a more nuanced picture has emerged, with T-type channels also playing a role in Ca^{2+} delivery for constriction over negative membrane potentials (Ca$_V$3.1/Ca$_V$3.3) or in privileged communication to large-conductance, Ca^{2+}-sensitive K$^+$ (BK) channels to oppose depolarization and constriction (Ca$_V$3.2; Harraz et al., 2014). Thus, the triumvirate of Ca$_V$1.2, Ca$_V$3.2, and Ca$_V$3.3 (or Ca$_V$3.1 in rats) channels appears to orchestrate the myogenic response to increases in intravascular pressure. However, the majority of these studies have used cerebral arteries from rodents. The specific expression, function, and contribution of Ca$_V$ channels to arterial tone in human arterial smooth muscle are not known.

Harraz et al. (2015) test a key assumption in the field: that Ca$_V$ function in rodent arterial smooth muscle of the cerebral circulation is a valid model for assessing Ca$_V$ function in human arterial smooth muscle in the brain. In this comprehensive study using human cerebral arteries, the authors determine the composition of Ca$_V$ isoforms, their functional properties, and their contribution to myogenic tone.

Ca$_V$ channel pharmacology and significance in human disease

Ca$_V$ channels are five-subunit complexes composed of a pore-forming α1 subunit and accessory α2, β, δ, and γ subunits. Ca$_V$ channels are segregated into different types depending on Ca^{2+} current properties of the α1 subunit, exemplified by L-type ("long lasting") Ca$_V$1.1, 1.2, 1.3, and 1.4 channels and T-type ("transient") Ca$_V$3.1, 3.2, and 3.3 channels. L- and T-type Ca$_V$ channels exhibit different activation and inactivation properties, with L-type channels displaying an activation curve shifted to more positive voltages (Perez-Reyes, 2003; Catterall, 2011). A rich pharmacology is available for manipulating L-type channels, including several therapeutic drugs such as dihydropyridines (e.g., nifedipine) that are capable of selectively inhibiting the channel. Comparatively speaking, pharmacological options for manipulating T-type channels are very limited. One drug, mibefradil, was initially touted as a selective T-type channel antagonist and made a brief appearance on the market before potentially serious drug interactions made it clear that the drug was not selective (Bezprozvanny and Tsien, 1995; Stolberg, 1998).

Ca$_V$ channels are involved in various human diseases. L-type Ca$_V$1 channels have been implicated in Timothy syndrome, cardiac arrhythmias, and autism spectrum disorders, and T-type Ca$_V$3 channels are involved in absence seizures, an inherited epileptic syndrome. Overexpression of Ca$_V$ channels has also been shown to play a role in cancer progression and peripheral diabetic neuropathy (Nelson et al., 2006; Catterall, 2011; Dziegielewska et al., 2014).

Ca$_V$ function in human cerebral vasculature

Much of what is known about the structure, function, and expression of Ca$_V$ channels comes from experiments in rodent models, begging the question: do human cerebral arteries behave similarly to cerebral arteries from rodents? Making judicious use of the meager pharmacological tools available (NNC 55-0396 indiscriminately blocks all T-type channel subtypes and is nonselective at higher concentrations, whereas nickel at 50 µM appears to be selective for Ca$_V$3.2 channels [Harraz et al., 2014]), Harraz et al. (2015) set out to

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uncover the functional role of Ca$_V$ subtypes in regulating arterial tone in human cerebral arteries. These functional analyses were complemented by molecular and biochemical techniques to determine human Ca$_V$ expression at mRNA and protein levels. All of these experiments used surface (pial) cerebral arteries collected from patients undergoing resection surgery to remove a tumor (glioblastoma or metastasis from another site) or alleviate various forms of epilepsy or seizure.

Directly comparing channel properties in isolated smooth muscle cells (SMCs) from humans, rats, and mice, Harraz et al. (2015) found that inward Ba^{2+} current density was notably smaller in human SMCs than in rodents. However, the current–voltage relationships of normalized maximal currents were similar, suggesting a common molecular basis. Similar leftward shifts in activation and inactivation curves were seen before and after application of nifedipine, indicative of the presence of both L- and T-type currents in human, mouse, and rat SMCs. Moreover, myogenic responses of human cerebral arteries were similar to previously published findings on rodents (Knot and Nelson, 1998).

Based on the relationship between pressure and diameter in the presence of nifedipine (L-type inhibition) and NNC 55-0396 (T-type inhibition) and the absence of external Ca^{2+}, to minimize Ca^{2+} entry through all pathways, the authors concluded that L- and T-type channels contribute similarly to tone at 20 and 40 mm Hg, whereas L-type channels dominate at pressures of 60–100 mm Hg. It had been previously shown that graded elevation of intravascular pressure from 20 to 100 mm Hg causes a graded membrane potential depolarization

of rat pial arteries from about −60 to −36 mV (Knot and Nelson, 1998), with 40 mm Hg corresponding to about −53 mV. Assuming that the membrane potential of human pial arteries is similar to that of rat arteries, these data collectively suggest that T-type channels have a significant role over a membrane potential range of −60 to −50 mV, a conclusion consistent with results of the authors' previous study using rodent cerebral arteries (Harraz et al., 2014). One notable difference between the human and rodent findings was that human cerebral arteries use Ca$_V$3.3, and not Ca$_V$3.1, to accomplish this task. Paradoxically, however, Ca$_V$3.2 exerted opposing effects. Harraz et al. (2015) provide evidence that Ca$_V$3.2 typically promotes vasodilation through a Ca^{2+} spark, BK-dependent mechanism (Fig. 1; Harraz et al., 2014). This suggests that the consequences of T-type channel suppression may vary depending on the relative contribution of Ca$_V$3.3 and Ca$_V$3.2 channels.

Intravital microscopy and in vivo measurements of vessel diameter and red blood cell velocity can be readily applied to animal models but are not generally suited to human experimentation. To circumvent this limitation, Harraz et al. (2015) generated a computer model to mimic a native cerebral arterial surface network based on the architecture, pressurized vessel myography, and electrophysiological data obtained from human tissue. The resulting model provides a starting point for predicting changes in blood flow that will become increasingly useful as more is understood about the degree to which Ca$_V$3.3 and Ca$_V$3.2 promote or inhibit arterial tone depending on the relative subtype dominance in a given tissue bed. As this information becomes available for various diseases, it may be possible to use the model to discern the appropriate balance of L-type– and T-type–specific drugs necessary to "tune" Ca$_V$ activity in a given tissue bed.
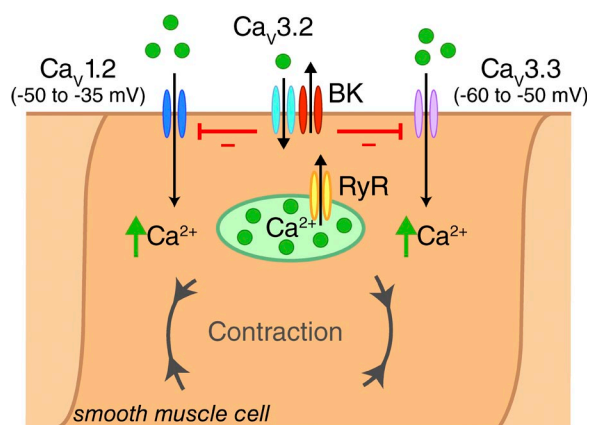
Paving the way for future studies on human cerebral vasculature

The work of Harraz et al. (2015) accomplishes two important tasks. First, and foremost, it is an impressively detailed study covering a broad range of approaches that include molecular analyses, tissue function, native channel function, and computer modeling in the most difficult of experimental systems: humans. Second, the caveats of the study, mainly its limited, heterogeneous subject population, highlight just how powerful rodent models can be for understanding human disease. By providing the ability to control age, genetic and environmental variability, and sample size, rodent models provide an invaluable tool for testing hypotheses in living systems that cannot otherwise be tested in vitro or in silico. It is notable how much of the data are in agreement with previously reported data from rodent models. In particular, the work of Harraz et al. (2015) in human tissue provides important conformation of their previous findings on divergent roles of Ca$_V$3.1 and Ca$_V$3.2 in rat

Figure 1. Model of the contribution of Ca$_V$ channels to smooth muscle tone. Ca$_V$1.2 (dark blue) and Ca$_V$3.3 (purple) promote smooth muscle contraction by allowing Ca^{2+} entry. The relative contribution of each depends on intraluminal pressure (or membrane potential). Ca$_V$1.2 is the major contributor between −50 and −35 mV (higher pressure, >40 mm Hg) whereas Ca$_V$3.3 is the major contributor between −60 and −50 mV (lower pressure, 20–40 mm Hg). Ca$_V$3.2 (light blue) opposes contraction through activation of Ca^{2+} sparks/RyRs through BK channel–dependent membrane hyperpolarization. Model is based on the data of Harraz et al. (2015).

cerebral arteries (Harraz et al., 2014), which may have therapeutic implications.

Out of necessity, most investigations on Ca_V channels have used Ba^{2+} as a charge carrier to increase current amplitude and have used conventional voltage-clamp protocols to examine activation and inactivation properties. In typical experiments on myogenic tone, pressure is stepped to different levels and held for minutes. Thus, exploring Ca_V properties under conditions similar to these would require measurement of steady-state currents, which, because of slow inactivation, are likely to be different from those inferred from short conditioning voltage pulses (Rubart et al., 1996). Ca^{2+}-dependent inactivation, which is absent in experiments with Ba^{2+}, provides an additional complicating factor. To better simulate physiology, it would therefore be important to measure steady-state Ca^{2+} currents through $Ca_V 1.2$, $Ca_V 3.2$, and $Ca_V 3.3$ channels over the membrane potential range (-60 to -36 mV) encompassed by intraluminal pressures of 20–100 mm Hg. Moreover, in vivo membrane potential likely continuously changes in response to the change in intraluminal pressure with each heartbeat. In mice, mean arterial pressure cycles every $\sim$100 ms, whereas human mean arterial pressure cycles every $\sim$1,000 ms. Therefore, it is tempting to speculate that differences in the kinetics of $Ca_V 3.3$ (human) and $Ca_V 3.1$ (rodent) are responsible for accommodating differences in pulse-pressure profiles in humans and rodents.

The proposed orchestration of Ca^{2+} influx into cerebral artery SMCs by $Ca_V 1.2$, $Ca_V 3.3$, and $Ca_V 3.2$ channels, which supply Ca^{2+} over different pressure/membrane potential ranges ($Ca_V 1.2$ and $Ca_V 3.3$) and preferentially activate BK channels ($Ca_V 3.2$), suggests a marvel of subspecialization (Fig. 1). Understanding how $Ca_V 3.2$ can preferentially communicate with BK channels will be critical to understanding their regulation of cerebral artery tone. Based on the ability of Ni^{2+} to inhibit Ca^{2+} spark–activated BK currents, it would appear that $Ca_V 3.2$ channels reside in a privileged Ca^{2+} communication complex with ryanodine receptors (RyRs) in the sarcoplasmic reticulum and nearby surface membrane BK channels (Harraz et al., 2014), an arrangement reminiscent of that described for TRPV4 channels–RyRs–BK channels in rat cerebral arteries (Earley et al., 2005). This inferred "nanostructure" cries out for further exploration using super-resolution microscopy. The observed opposing roles of $Ca_V 3.2$ and $Ca_V 3.3$ in human cerebral arteries dictates that therapeutic interventions will ultimately require $Ca_V 3$ subtype–specific modulators, elevating an already challenging pharmacology (Fig. 1).

Studies on human cerebral arteries are constrained by several limitations, including small sample size, heterogeneous populations, and difficulty of obtaining material from healthy individuals. Champions of translational research will laud the basic science rigor with which

Harraz et al. (2015) directly approach human physiology. Those with a more basic science bent may bemoan the fact that the tissues were obtained from a small sample of subjects with diseases that may have been the result of perturbations of Ca_V function or expression. Ultimately, the importance of performing basic research on human tissue takes precedence over such concerns, legitimate though they may be. In the case of the study by Harraz et al. (2015), the novelty of the findings presented is certain to pave the way for future studies.

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