T-Type Calcium Current in Sickle Cell Disease
A Channel to Therapy?

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In 1910, James Herrick made the first report of a case of sickle cell anemia. He described thin, elongated, sickle-shaped, and crescent-shaped red corpuscles. Soon afterward, the observation of sickle cells in the asymptomatic father of a sickle cell anemia patient raised the possibility of an inherited disorder. The distinction between symptomatic sickle cell anemia and the asymptomatic sickle cell trait was established in 1933. The difference between normal hemoglobin (HbAA) and sickle hemoglobin (HbSS) was recognized in 1959 to be the substitution of a valine residue for a glutamic acid in the β-chain amino terminus. Patients who have the sickle cell trait are heterozygotes (HbAS), having an abnormal, as well as a normal, β-globin gene. In sickle cell anemia, sickling may start at an oxygen saturation as high as 85%, while in the trait the desaturation has to be more severe before sickling is induced.

Sickling leading to vaso-occlusion and infarction occurs in many organs but was first described in the lungs. Initially it was thought that deformation and increased rigidity of the erythrocytes, related to polymerization of HbSS, was sufficient to cause mechanical obstruction. However, the work of Hebbel et al. focused attention on erythrocyte adherence to the endothelium as a mechanism promoting microvascular occlusion. A key observation in this regard is that sickle erythrocytes adhere more readily to microvascular endothelium than to endothelium from conduit vessels. If endothelial cells circulating in the blood can be taken as representative of the sedentary population, the endothelium is activated in sickle cell patients, whether in steady state or in an acute crisis. This is shown by expression of the adhesion molecules, ICAM-1, VCAM-1, E-selectin, and P-selectin.

More recent work has implicated inflammation as a factor in the pathogenesis of vaso-occlusive crises. An increased leukocyte count is an independent risk factor for such episodes, and activated neutrophils have been demonstrated to increase retention of sickle erythrocytes in the lung. A two-stage model has been proposed, comprising increased adhesion of cells to the endothelium and subsequent mechanical obstruction. Although many signaling molecules are involved in cell adhesion, thrombin plays a major role in the chronic inflammation seen in sickle cell disease and particularly in endothelial cell activation. Human umbilical vein endothelial cells (HUVECs) exposed to thrombin in vitro show increased adhesivity for sickle cells, while at the same time demonstrating significant interendothelial cell gap formation.

Thrombin, by stimulating G protein (Gq)-coupled protease-activated receptors (PARs), increases cellular diacylglycerol (DAG) and inositol trisphosphate (IP3), the latter causing calcium release from the sarcoplasmic and endoplasmic reticulum (SER) (see Figure). Increased DAG and depletion of the SER stores activate store-operated channels (SOCs) (in the plasmalemma) permeable to calcium, triggering further entry of calcium into the cell, activation of myosin light chain kinase (MLCK), and thus cellular contraction. Interendothelial cell contraction results in the formation of gaps between cells in the endothelium. This gap formation is enhanced by a decrease in cyclic adenosine monophosphate (cAMP). cAMP is the crucial signal that normally promotes interendothelial cell adhesion. The two second messengers, calcium and cAMP, while responsible for contraction and adhesion, respectively, influence each other in the endothelium. On the one hand, SOCs colocalize with calcium-sensitive adenylate cyclases and the increased calcium entry through SOCs decreases cellular cAMP levels, partly by inhibiting type 6 adenylate cyclases. On the other hand, cAMP inactivates MLCK and consequently inhibits contraction. In addition to intracellular calcium, thrombin may enhance gap formation through calcium-independent proteolytic pathways.

The lung microvasculature arises through vasculogenesis while angiogenesis gives rise to the macrocirculation. As a consequence, endothelial cells in the adult lung retain different phenotypes: macrovascular endothelial cells produce more nitric oxide than their microvascular counterparts; activation of SOCs and a rise in cytosolic calcium cause leaks in the macrovasculature barrier leaving the microvasculature relatively unscathed. Although both pulmonary artery endothelial cells (PAECs) and pulmonary microvascular endothelial cells (PMVECs) possess SOCs, stimulation of calcium entry through SOCs results in a differential effect in PAECs versus PMVECs. Whereas thapsigargin (a blocker of the SER calcium ATPase and therefore an activator of SOCs) induces shape change and gap formation in PAECs, PMVECs appear resistant to such perturbations. This difference in the effect of calcium entry has been attributed to the tighter regulation of cAMP levels in PMVECs. Finally, while PMVECs have been observed to have a resting membrane potential centered at −22 mV, PAECs have a bimodal distribution of resting membrane potentials centered at −32 and −60 mV.
Thrombin binds to protease-activated receptors in the plasma-lemma, triggering a Gα-linked increase in calcium-dependent potassium current (KCa). This causes membrane hyperpolarization, which activates cyclic nucleotide-gated (CNG) channels causing depolarization into the range of potentials at which T-type (CaV3.1) calcium currents are active. Thrombin also causes release of calcium from the sarcoplasmic reticulum (SR) via an IP3 pathway; depletion of SR stores activates a store-operated channel (SOC) that is permeable to calcium. These two sources of calcium trigger contraction of the cell.

Potentials peaking at $-63$ and $-24$ mV.20 This piece of information makes the data of Wu et al,21 in this issue of Circulation Research, especially intriguing: they show that the ion channel responsible for thrombin-mediated increases in the cytosolic calcium of lung microcirculation endothelial cells is the T-type voltage-dependent calcium channel. T-type calcium channels are normally associated with excitable cells that have resting potentials close to the reversal potential for potassium ions (typically $-85$ mV). Consequently, T-type channels are usually activated by depolarizing stimuli. In the case of PMVECs, with a resting potential close to $-20$ mV,20 activation of T-type channels occurs when thrombin causes an initial hyperpolarization by opening calcium-sensitive potassium channels, followed by depolarization due to cyclic nucleotide-gated ion channels into the range of voltages in which T-type currents are active.

Most endothelial cell types do not have voltage-gated calcium channels and the lack of L-type calcium channels in the macrovasculature endothelium is well established. However, the suggestion that capillary endothelial cells do have voltage-gated calcium channels22–24 predates the molecular identification of T-type voltage-gated calcium channels. It is now known that there are three T-type calcium channel genes: CaV3.1 (α1I), CaV3.2 (α1II), and CaV3.3 (α1III).25 While the related Ca1 and Ca2 subfamilies (responsible for the L-, N-, P-, and Q-type calcium channel currents) of voltage-gated calcium channels require auxiliary subunits to recapitulate native channel properties, CaV3 channels appear to be self-sufficient. Of the three classes of auxiliary subunits, αδ, β, and γ, increase CaV3 currents with minor effects on kinetics, and γy, γc, and γv subunits accelerate inactivation. To date, there are no known auxiliary subunit effects on CaV3 channel activation, indicating that the shift in the voltage dependence of steady-state inactivation of PMVECs seen by Wu et al21 could be due to a splice variant. Calcium entry through T-type channels has a large role in the “bursting” activity of neurons and of neuroendocrine cells. This is attributed to the very negative potentials ($-60$ to $-20$ mV) at which T-type channels activate (and hence their other name, LVA, or low voltage-activated channels) and the fast activation and inactivation (and hence the “T” for transient) kinetics of these channels. Thus, the role of T-type calcium channels in calcium entry in a nonexcitable cell type such as endothelial cells is even more surprising.

Although the lack of high-affinity antibodies to CaV3 protein has hampered efforts so far to reveal localized expression patterns, it has been shown that CaV3.1 is expressed primarily in the brain, heart, ovary, and placenta. Notably, significant expression of CaV3.1 is seen in the fetal kidney and lung as well as in adult kidney tubules. This, together with evidence that CaV3.2 is present in renal artery smooth muscle cells, may explain the antihypertensive effect of the T-type calcium channel blocker mibefradil.26 The use of specific antibodies recently designed27 should reveal the distribution of T-type channels within the lung.

The results of Wu et al21 raise a number of exciting questions: What other microcirculation endothelial cells express T-type channels or other voltage-gated calcium channels? Mortality in patients with sickle cell disease is high, with median ages of 42 (male) and 48 (female) at time of death.28 One third of deaths attributable to sickle cell disease are caused by acute crises (pain, chest syndrome, and stroke). Can targeted delivery of T-type blockers be used as therapy for acute chest syndrome? If T-type channels are present in the spleen and brain vessels, could T-type channel blockers also prevent infarction in these organs?

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