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TRPM8 Contributes to Early Temperature-Induced Platelet Activation

Anastasiia Stratiievska[1], Olga Filippova[2], Hasan Tahsin Ozpolat, MD[3], Daire Byrne[1], S. Lawrence Bailey[2], Molly Mollica, PhD[4], Jeffrey Harris[5], Kali Esancy[6], Ajay Daka[6], Nathan Sniadecki, PhD[7], Jose A. Lopez, MD[8], Moritz Stolla, MD[9,10,11]

[1] Bloodworks NW Research Institute, Seattle, WA
[2] Bloodworks NW Research Institute, Seattle
[3] Department of Medicine, University of Arizona, Tucson, AZ
[4] Bloodworks Northwest, Seattle, WA
[5] Bloodworks Research Institute, Seattle, WA
[6] Department of Biological Structure, University of Washington, Seattle
[7] Mechanical Engineering, Bioengineering, University of Washington, Seattle, WA
[8] University of Washington, Bloodworks Research Institute, Seattle, WA
[9] Thomas Jefferson University, Seattle, WA
[10] Bloodworks Northwest Research Institute, Seattle, WA
[11] Department of Medicine, Division of Hematology, University of Washington, Seattle, WA

Background: Like sensory neurons, platelets are activated by a decrease in temperature. However, the molecular mechanism of this temperature-sensing ability is unknown. Platelet activation by temperature could contribute to numerous clinical observations, most importantly to the well-known reduced lifespan of *ex vivo*-stored platelets for transfusion. In this study, we sought to dissect the molecular basis of the cold-induced platelet activation response and specifically, evaluate the role of the thermosensitive ion channel transient receptor potential cation channel subfamily member 8 (TRPM8) in platelets and their precursor cells.
Methods: We tested for the presence of TRPM8 on the megakaryocytic cell line MEG-01 and human platelets by immunoblotting, confocal microscopy, and imaging flow cytometry. The effect of TRPM8 on platelets was evaluated using the commercially available TRPM8 inhibitor PF-05105679. In addition, we used TRPM8 agonists such as menthol, WS-12, and icilin. Platelet shape change and αIIbβ3 integrin activation were assessed by imaging flow cytometry and conventional flow cytometry. We tested for platelet aggregation using light transmission aggregometry and for intracellular calcium by reading fluorescence on a plate reader after loading platelets with Fura-2-AM. Calcium release in response to temperature decrease was assessed after loading platelets or TRPM8-transfected HEK293T/17 and reading fluorescence in a Real-Time PCR Detection System.
Results: We detected TRPM8 mRNA and protein in MEG-01 cells and platelets but there was a remarkable variability among donors. Further evidence for TRPM8's presence in platelets came from the fact that inhibitors of TRPM8 prevented early temperature-induced platelet activation events, such as αIIbβ3 integrin activation and shape change. Unlike temperature changes, chemical agonists of TRPM8 did not seem to have an acute effect on platelets. Platelets exposed to below-normal body temperature displayed a cytosolic calcium increase, but this was independent of TRPM8 and entirely dependent on the calcium release from the endoplasmic reticulum.
Conclusion: We found evidence for TRPM8 expression in platelets but the expression varies greatly between individuals and is of unclear physiologic significance. Our study suggests that the cold response of platelets is complex, and TRPM8 appears to play a calcium-independent role in the early temperature-induced activation of platelets, while other mechanisms likely contribute to later stages of temperature-mediated platelet response. Because of the high interindividual variability of TRPM8 expression, a population-based approach should be the focus of future studies.

Disclosures No relevant conflicts of interest to declare.

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