Possible Calcium-Independent Nitric Oxide Production Triggered by Sphingomyelinase in Red Blood Cells

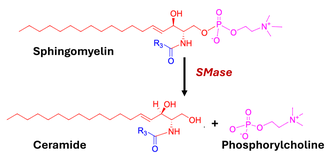
Nishaka William[1] and Jason P. Acker[1,2]

[1]Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, Alberta, T6G 2R3, Canada
[2]Innovation and Portfolio Management, Canadian Blood Services, 8249 114th Street, Edmonton, Alberta, T6G 2R8, Canada

Background

Overview of Sphingomyelinase (SMase):

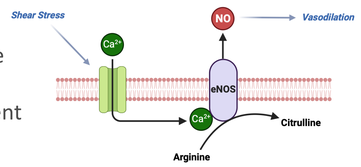
- SMase is responsible for converting sphingomyelin into the signaling lipid, ceramide
- Because ceramide is heavily involved recruitment and activation of immune cells, SMase levels are heightened during inflammation
- In RBCs, ceramide is predominantly involved in induction of eryptosis



SMase-Catalyzed Reaction

Overview of endothelial nitric oxide synthase (eNOS):

- eNOS is responsible for regulating vascular tone through the production of nitric oxide (NO)
- In RBCs, eNOS activation is thought to be primarily dependent on an acute rise in intracellular calcium



Shear-Induced Vasodilation

The link between SMase and eNOS:

- Conversion of sphingomyelin into ceramide causes displacement of eNOS from lipid rafts, compromising inhibitory interactions between eNOS and membrane proteins

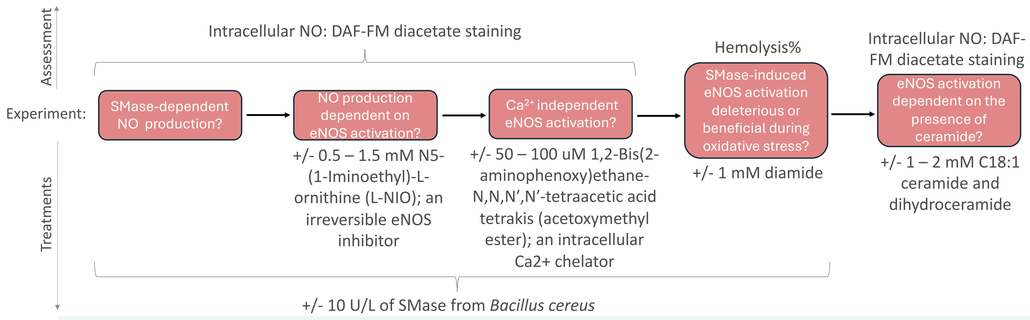
The relevance of calcium-independent mechanisms of eNOS activation: The cell is able to rapidly release intracellular calcium, making calcium-dependent eNOS activation mechanisms very transient. Calcium-independent mechanisms are likely to be more sustained.

Hypothesis and Major Questions

The activity of SMase and loss of inhibitory raft interactions can lead to aberrant eNOS activity that does not require a rise in intracellular calcium

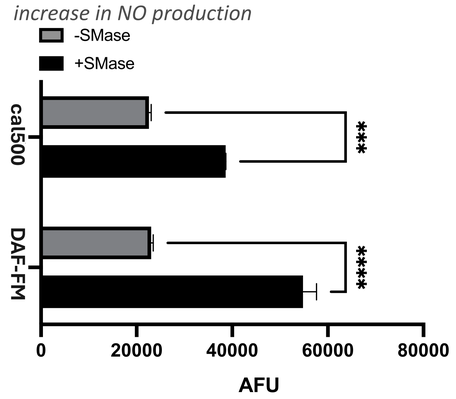
1. Does treatment with SMase cause an increase in eNOS-dependent NO production, and if so, is this dependent of calcium?
2. Are changes in NO production upon ceramide treatment specifically dependent on the conversion of membrane sphingomyelin into ceramide or ceramide itself?
3. Are the changes in NO production adaptive or deleterious to the cell?

Materials and Methods

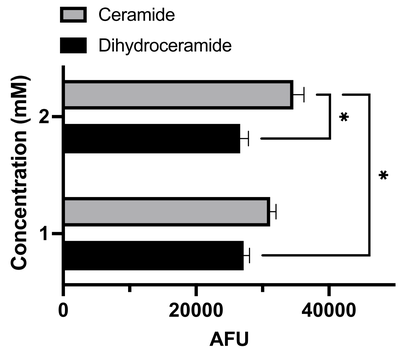


Results

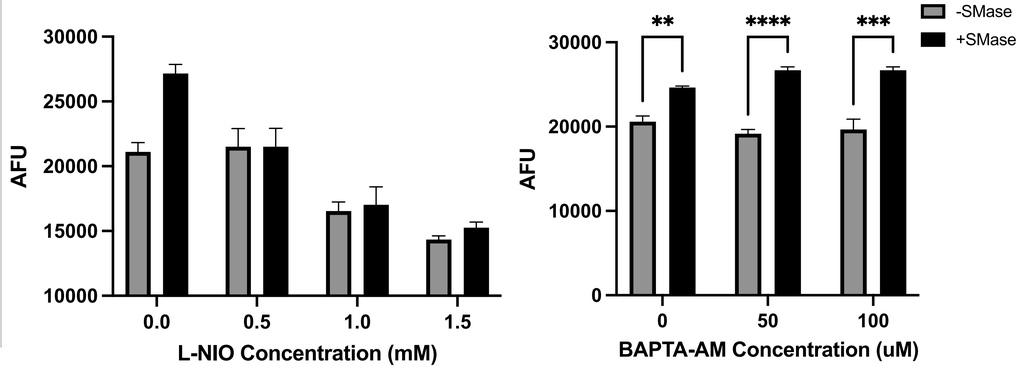
SMase causes an influx of calcium and an increase in NO production



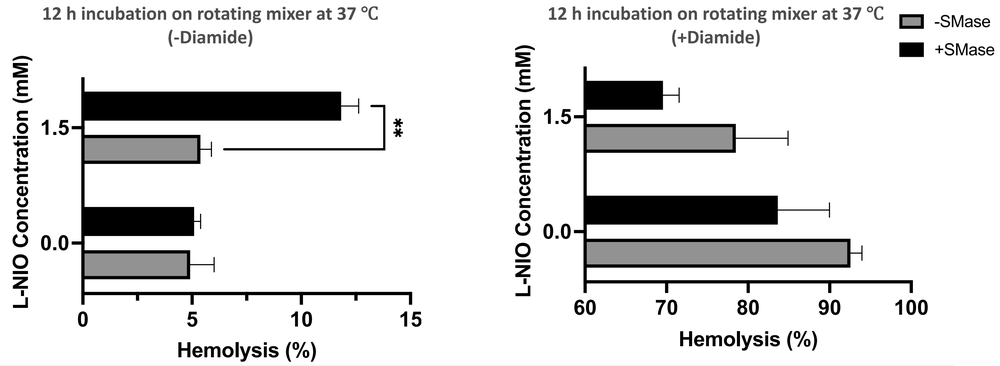
SMase-dependent NO production is dependent on the increase in ceramide



The increase in NO Production is dependent on eNOS and independent of the rise in the intracellular calcium concentration



SMase-dependent NO production may offer protective effects against RBCs under conditions of minimal oxidative stress; however, it does not appear to contribute to cellular injury when oxidative stress is significant



Conclusions and Future Outlook

Conclusions:

1. Treatment of RBCs with exogenous SMase causes an influx of calcium, but also activates eNOS in a calcium-independent manner.
2. SMase-dependent activation of eNOS is dependent on the accumulation of ceramide, rather than just the loss of sphingomyelin
3. When oxidative stress is minimal, there may be some protection imparted by SMase-dependent activation of sphingomyelin

Future questions to be addressed:

1. What is the relevance of SMase-dependent eNOS activation under inflammatory conditions characterized by a rise in SMase and how 'sustained' is the calcium-dependent mechanism of activation relative to canonical activation mechanisms?
2. How is the lipid composition of the membrane at the time exposure to exogenous SMase related to eNOS activation?
3. What is the interaction between ceramide-mediated eNOS activation and other injury mechanisms caused by ceramide accumulation?

Acknowledgements and Conflicts of Interest

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