Mechanosensor PIEZO1-mediated endothelial nitric oxide synthase phosphorylation. Is WNK the missing link?

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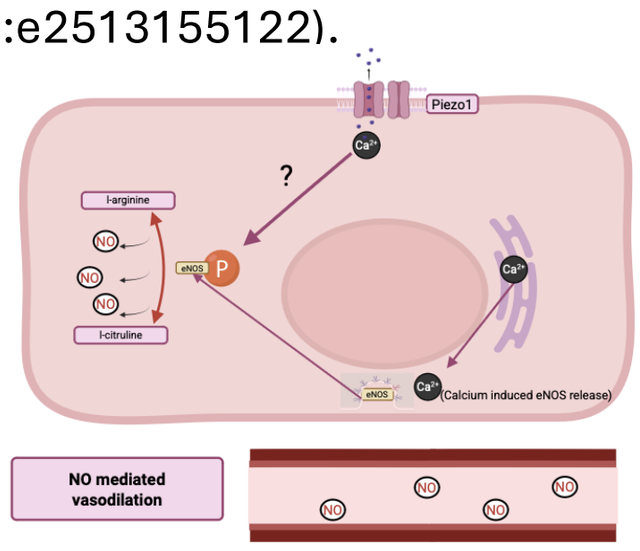
Background

PIEZO1 is a mechanosensor expressed in human umbilical vein endothelial cells (HUVECs). We found its activation results in rapid endothelial nitric oxide synthase (eNOS) phosphorylation at serine site 1177 (Ser1177) independent of protein kinase B (AKT) (Debant et al in preparation).
The serine-threonine kinase linked with this rapid PIEZO1-mediated eNOS phosphorylation is unknown. Further understanding could provide insight into potential future therapeutics to enhance placental blood flow in conditions such as fetal growth restriction where placental blood flow is impaired.
This study aimed to investigate with no lysine kinases (WNK1-4) as responsible candidates in this pathway because of their recently suggested relevance to PIEZO1 (Jung JU, Stippec S, Cobb MH. Proc Natl Acad Sci U S A. 2025 Sep 2;122(35):e2513155122).



(Image created with BioRender.com)

Methods

Cell culture and treatment

For inhibitor experiments, HUVECs were cultured and treated with and without WNK1-4 and WNK1 specific inhibitors (1μM) for 30 minutes prior to experimental techniques.
To achieve WNK1 knock-down, HUVECs were transfected with non-targeting control siRNA or WNK1 target-specific siRNA 48 hours prior to experiments.

Western blotting

HUVECs were stimulated with PIEZO1 agonist Yoda1(Y1) (2μM) for 1 minute or dimethyl sulphoxide (veh) as control for inhibitor experiments.
For WNK1 knock-down experiments, HUVECs were treated with veh, Y1 (2μM) , Y1 (5μM), alternative agonist Yoda2 (Y2) (2μM) or ionomycin (10uM).
Following protein lysate extraction, gel electrophoresis and membrane transfer, membranes were incubated with anti-eNOS-pS1177 and anti-total eNOS or anti-WNK1antibody prior to imaging with iBright™ imaging software.

Calcium flux assay

To investigate the impact of WNK 1-4 and WNK1 inhibitors on PIEZO1 activity, a calcium flux assay was undertaken with fura-2 and Flexstation®3.

Statistical analysis

The t-test was used and analysis performed using Prism (v11.0.0).

Results

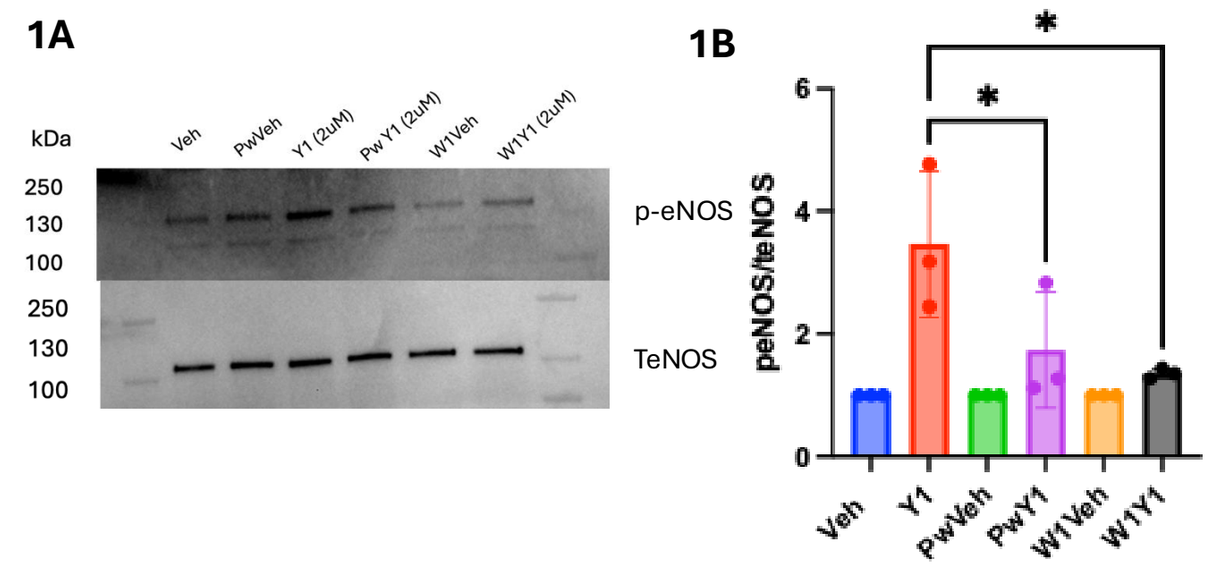


Figure 1. PIEZO1-mediated eNOS phosphorylation is reduced by WNK1-4 and WNK1 inhibitors Figure 1A a representative Western blot suggesting reduced Y1-evoked eNOS phosphorylation after treatment with WNK1-4 (Pw) and WNK1 (W1) inhibitors. Figure 1B suggests reductions in Y1 evoked eNOS phosphorylation following treatment with the inhibitors ($p<0.05$, n=3 independent experiments).

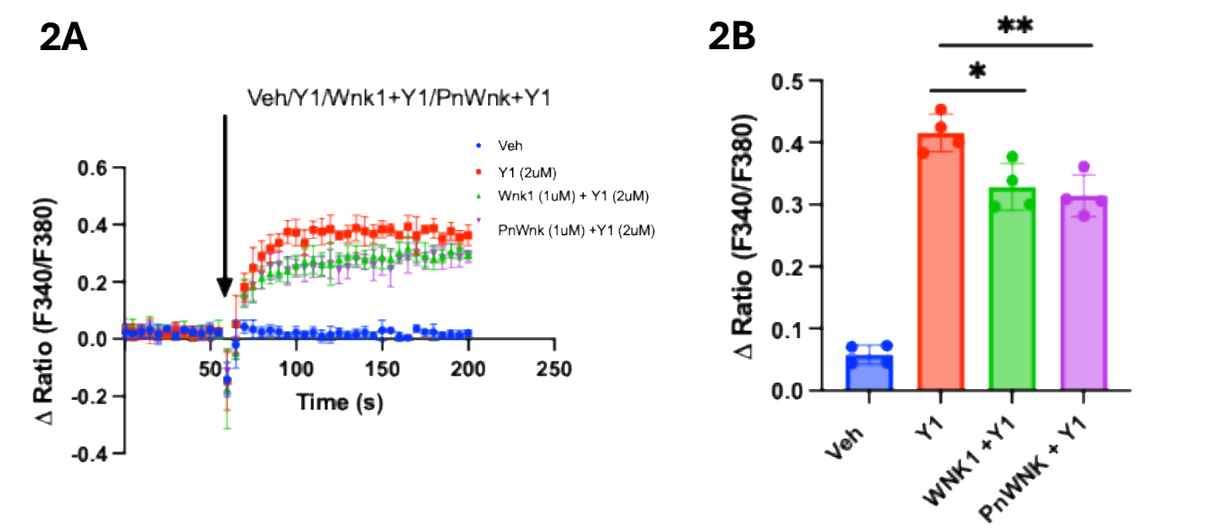


Figure 2. WNK1-4 and WNK1 inhibitors reduce Yoda1-mediated PIEZO1 calcium flux Figure 2A is a calcium flux assay demonstrating a reduction in calcium flux following stimulation with Y1 in the presence of WNK1 specific and WNK1-4 inhibitors. Figure 2B shows that this reduction is small but significant ($p<0.05$* and 0.01**).

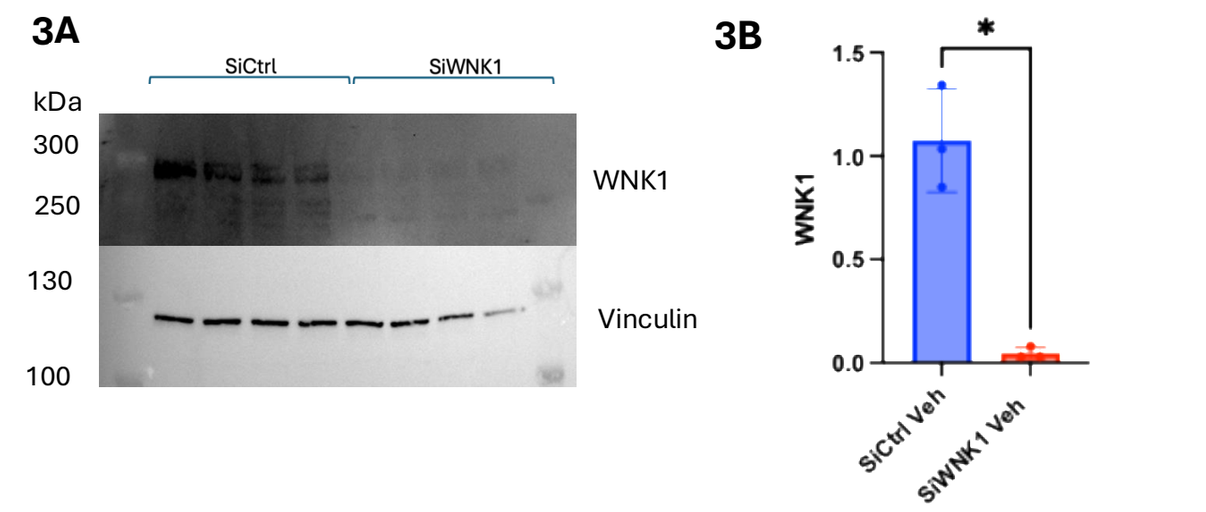


Figure 3. WNK1 is strongly depleted by WNK1 siRNA 3A a representative Western blot for siCtrl Vs siWNK1, vinculin as loading control. 3B shows the knock-down effect to be significant ($p<0.05$).

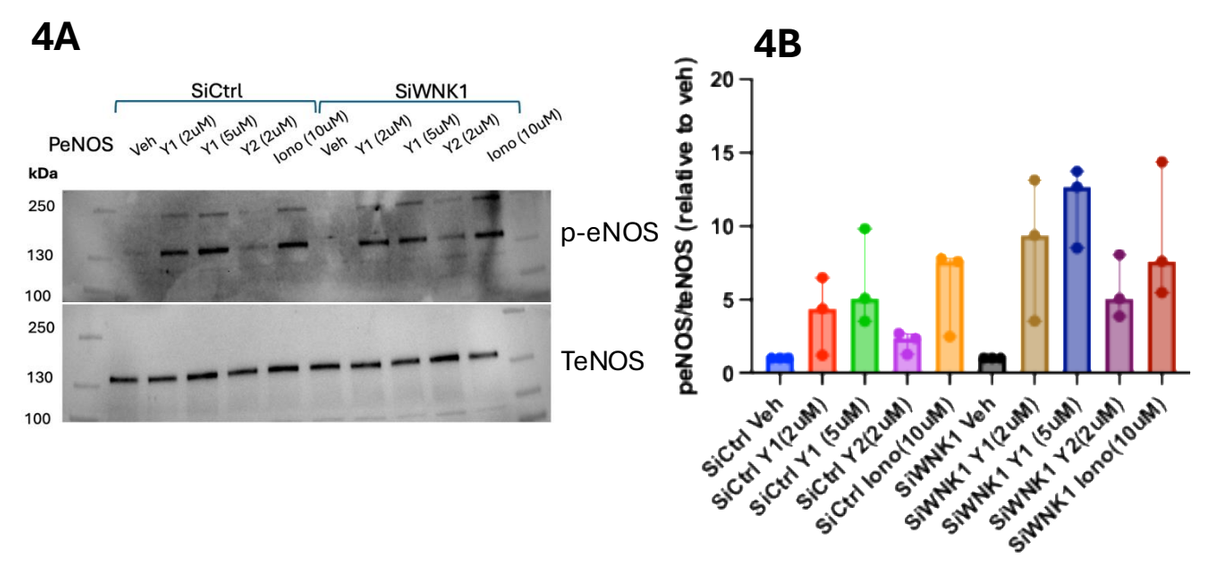


Figure 4. WNK1 knock-down does not alter PIEZO1-mediated eNOS phosphorylation 4A is a representative Western blot for eNOS phosphorylation with treatments veh, Y1, Y1, Y2, Iono (SiCtrl Vs SiWNK1). 4B demonstrates no significant difference in PIEZO1-mediated eNOS phosphorylation following WNK1 knock-down across treatment groups.

Conclusions

Findings of this study do not support WNK1 as the responsible serine-threonine kinase involved in rapid PIEZO1-mediated eNOS phosphorylation. Further work is therefore required to identify the responsible serine-threonine kinase involved in this pathway.

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