Characterization of a novel vasorelaxing peptide derived from the G Protein-coupled Estrogen Receptor 1

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BACKGROUND

- Vascular endothelial cells are the physiological source of nitric oxide (NO), which is crucial for the control of vascular tone, cardiac functions, and other beneficial actions in many organ systems.
- Many endothelial functions are dependent on store-operated Ca^{2+} entry (SOCE) (Fig. 1A). Herein, depletion of the endoplasmic reticular (ER) Ca^{2+} content triggers intra-ER conformational changes in the stromal interaction molecule 1 (STIM1) to promote its interaction with plasma membrane channels for Ca^{2+} entry (Fig. 1B). Sustained ER Ca^{2+} depletion, however, is associated with ER stress and endothelial dysfunction, a common feature of many pathological conditions such as hypertension and diabetes.
- Our laboratory previously identified a specific interaction between the intra-ER Ca^{2+}-binding loop of STIM1 and the G protein-coupled estrogen receptor (GPER), a receptor that mediates many cardiovascular protective functions (reviewed *Front Endocrinol 2020, PMID 33133016*). From these studies, we have developed a peptide (G2) based on the GPER-STIM1 interacting domain and conjugated it with an endothelium-specific leader sequence to generate a tandem peptide (EFG2). Due to the biochemical properties of the G2 domain, EFG2 is predicted to specifically promote endothelial Ca^{2+} entry and Ca^{2+}-dependent functions without emptying ER Ca^{2+}.

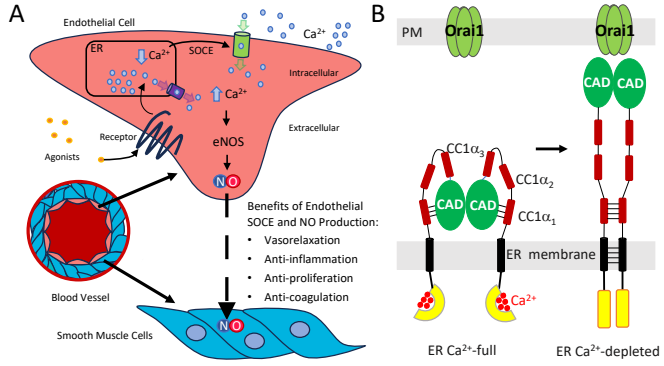

Figure 1. Role and mechanisms of SOCE in endothelial NO production and vasorelaxation. *A, Benefits of endothelial SOCE and consequent NO production.* ***B,*** *Molecular mechanism of STIM1 involvement in activation of SOCE. PM, plasma membrane; ER, endoplasmic reticulum; CC1, coiled-coiled domain 1; CAD, CRAC [Ca^{2+} release-activating Ca^{2+} entry]-activating domain; Orai1, a key store-operated Ca^{2+} channel.*

HYPOTHESIS

By specifically targeting endothelial cells and binding STIM1's Ca^{2+}-binding loop with high affinity, EFG2 promotes STIM1's conformational changes that trigger Ca^{2+} entry in endothelial cells without causing ER Ca^{2+} release, thereby stimulating nitric oxide production and vasorelaxation.

METHODS & RESULTS

Design of FRET-based biosensors for STIM1's Ca^{2+}-binding loop

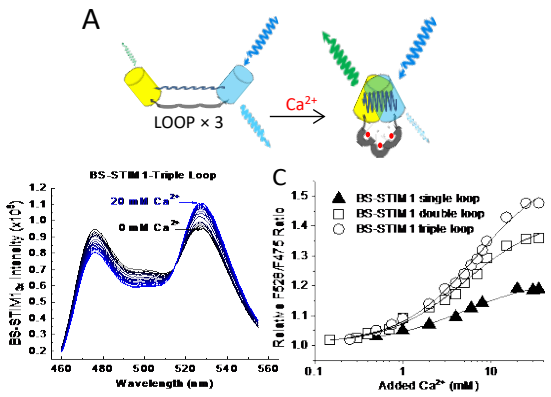

Figure 2. *A, BS-STIM1$_{3x}$ design (triple loop).* ***B,*** *Emission spectra of purified BS-STIM1$_{3x}$ upon Ca^{2+} titration. Purified biosensors were excited at λ430 nm.* ***C,*** *Ca^{2+} binding curves of single-, double- and triple-loop STIM1 biosensors.*

Specific interactions between EFG2 peptide and STIM1's Ca^{2+}-binding loop

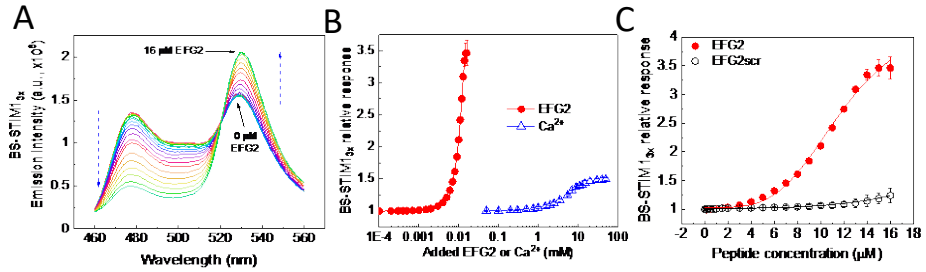

Figure 3. Direct interaction between EFG2 and STIM1's Ca^{2+}-binding loop. *A, Emission spectra of BS-STIM1$_{3x}$ upon incremental titration of EFG2.* ***B,*** *Biosensor response as a function of EFG2 or Ca^{2+} concentration.* ***C,*** *Biosensor response to EFG2 or EFG2$_{scr}$. N=4.*

Peptide EFG2 triggers Ca^{2+} entry in endothelial cells but not in vascular smooth muscle cells

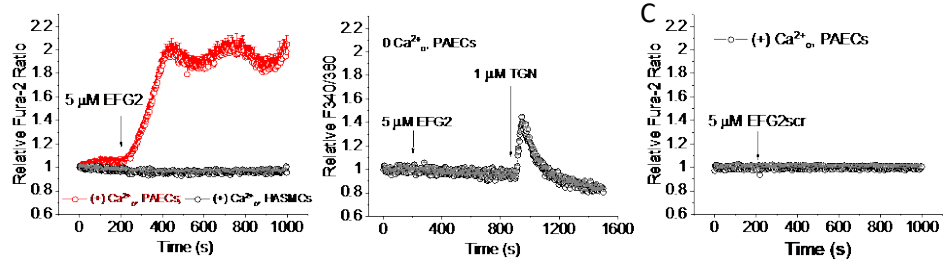

Figure 4. Effects of EFG2 on intracellular Ca^{2+} ($[Ca^{2+}]_i$) in primary porcine aortic endothelial cells (PAECs) and human aortic smooth muscle cells (HASMCs).
A, *Effects of EFG2 on $[Ca^{2+}]_i$ PAECs vs HASMCs in the presence of extracellular Ca^{2+}. Note the lack of effect from EFG2 on HASMCs, indicating the specificity of EFG2 to ECs.*

B, *Effects of EFG2 on ER Ca^{2+} stores in PAECs in the absence of extracellular Ca^{2+} followed by addition of SERCA inhibitr thapsigargin (TGN). Note the initial absence of EFG2 signal followed by the TGN-induced signal implying that the effect of EFG2 does not affect ER Ca^{2+} stores.*

C, *Effect of EFG2scr in PAECs in the presence of extracellular Ca^{2+}. Note the absence of an effect of EFG2scr in PAECs verifying the specificity of the G2 domain in triggering Ca^{2+} entry.*
Traces are averages ± SD of n = 35 – 73 cells from 3 – 6 repetitions for each condition.

G2 does not affect cardiac voltage-dependent Ca^{2+} current or cause endothelial ER stress

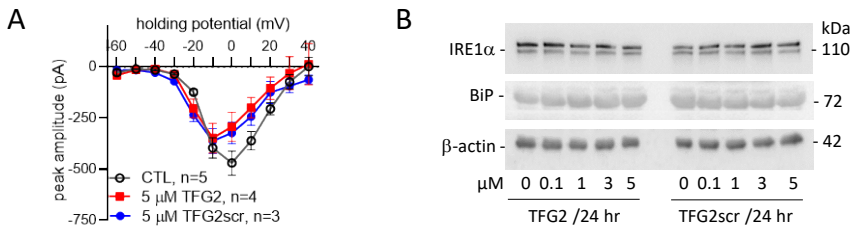

Figure 5. Effect of TFG2 and TFG2$_{scr}$ on $I_{Ca,L}$ in cardiomyocytes and ER stress markers IRE1α and BiP in PAECs. *G2 peptide and scrambled peptide is conjugated to a universal cell penetrating sequence (TAT) to generate TFG2 and TFG2scr.*

A, *Current-voltage relationships of L-type Ca^{2+} current ($I_{Ca,L}$) in freshly isolated cardiomyocytes pre-injected with vehicle (CTL) or specified concentration of TFG2 and TFG2$_{scr}$ via patch electrode tip. 5-min baseline/diffusion was allowed before data acquisition.*

B, *Sub-confluent PAECs were treated with the specified concentrations of TFG2 or TFG2scr before lysis and immunoblotting for the specified ER stress markers. N = 2.*

In vivo vasorelaxing effects of EFG2

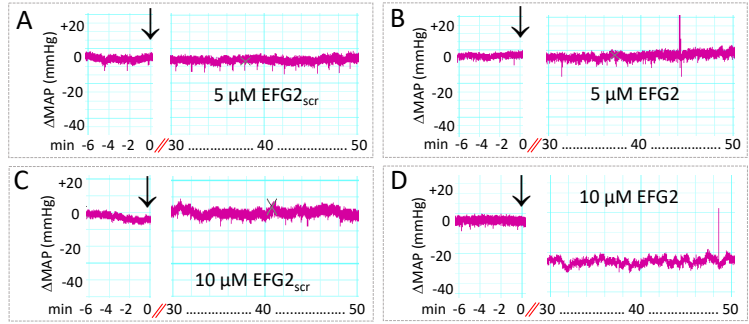

Figure 6. Effects of EFG2 and EFG2scr on intra-aortic blood pressure. *8-weekold male anesthetized Sprague-Dawley rats were given intravenous EFG2 or EFG2scr as arterial blood pressure was monitored.* ***A-D,*** *Changes in mean arterial pressure (ΔMAP) before and after injection of the specified peptide (arrows). N = 1 for each dose.*

Loss of sustained vasorelaxing effect when G2 is tagged with a universal cell penetrating sequence

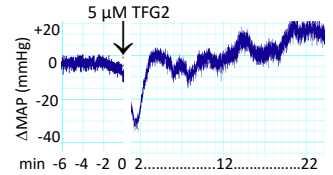

Figure 7. Effects of TFG2 on intra-aortic blood pressure. *Changes in mean arterial pressure (ΔMAP) before and after injection (arrow) of TFG2 in an 8-wk-old male Sprague-Dawley rat. Note the biphasic response consisting of an initial vasorelaxation response followed by rapid vasoconstriction.*

SUMMARY & CONCLUSIONS

- We successfully generated a biosensor that reports interactions of binding partners with the Ca^{2+}-binding loop of STIM1.
- The endothelium-specific, GPER-based EFG2 peptide directly interacts with the Ca^{2+}-binding loop of STIM1 with high affinity and produces a large conformational change in this domain, whereas the control peptide with scrambled G2 sequence (EFG2scr) has a projected 100-fold lower affinity.
- In primary endothelial cells, EFG2 triggers Ca^{2+} entry while the scrambled peptide does not. EFG2 does not trigger any Ca^{2+} entry in human aortic smooth muscle cells (HASMCs), which signifies that EFG2 is selective for endothelial cells.
- Using a universal cell-penetrating tag (TAT) we demonstrated that G2 does not affect voltage-dependent Ca2+ entry (in excitable cardiomyocytes) or cause endothelial ER stress.
- In anesthetized rats, intravenous infusion of EFG2 acutely causes sustained dose-dependent vasorelaxation compared to the scrambled peptide.
- When tagged to the universal cell-penetrating sequence, G2 produces a biphasic response. The initial vasorelaxation presumably reflects NO production from the endothelium. The subsequent gradual increase in MAP likely indicates vasoconstriction due to Ca^{2+} entry in the underlying VSMCs as TFG2 enters these cells. This response further supports the specificity of EFG2 for the endothelium.
- These data suggest that EFG2 can be developed as a mechanistically novel therapeutic agent to improve endothelial functions in conditions such as arterial hypertension and atherosclerosis.

ACKNOWLEDGMENTS

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