Spike Proteins Increase Endothelial Calcium Via TRPV4

****WINNER** Basic Science Category for 2022 Lifespan Research Day**

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Abstract

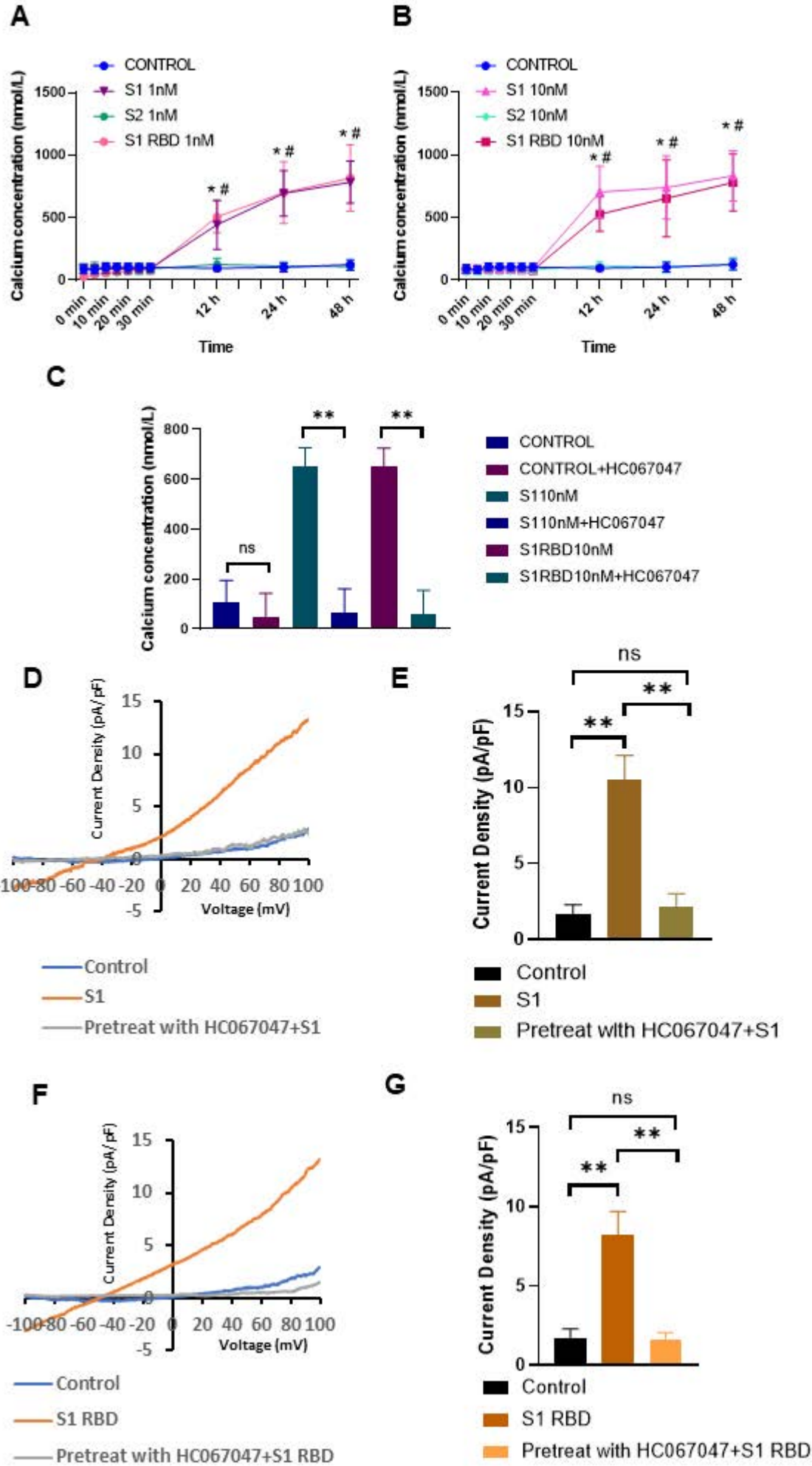
Background & Aim: Endothelial dysfunction plays a central role in the pathogenesis of acute respiratory distress syndrome (ARDS) with COVID−19. Transient receptor potential vanilloid 4 (TRPV4), a cation channel ubiquitously expressed, can regulate inflammatory cytokines that play key roles in acute lung injury/ARDS. However, it is unknown whether spike proteins can affect TRPV4 activity and related Ca2+ signaling in pulmonary microvascular endothelial cells. We hypothesized that spike protein causes activation of TRPV4 channels, resulting in increases in intracellular Ca2+, which may lead to pulmonary endothelial dysfunction.

Methods: Intracellular Ca2+ concentrations in human lung microvascular endothelial cells (HLMECs) were measured by calcium imaging in the presence of SARS CoV−2 Spike protein S1, receptor−binding domain (RBD) of S1, or protein S2 with or without co−incubation of the selective TRPV4 antagonist (HC−067047).

Results: The intracellular Ca2+ concentration of HLMECs was significantly increased when incubated with S1 (1nM, n=20; 10nM, n=24) or S1 RBD (1nM, n=18; 10nM, n=12) for 12, 24, 48 hours, relative to control (n=11) or S2 (1nM, n=10; 10nM, n=13) ($p<0.05$, Fig. A, B). Co−incubation of HC−067047 (500nM) significantly attenuated Ca2+ intracellular influx upon treatment with S1 (10nM, n= 64, 24 hours, $p<0.05$) or S1 RBD (10nM, n= 80, 24 hours, $p<0.05$) (Fig. C). TRPV4 sensitive current density was significantly increased when incubated with S1 (10nM, n=10) or S1 RBD (10nM, n=10) for 24 hours ($p<0.05$ vs. control, respectively, Fig. D−G), whereas co−incubated with HC−067047 (500nM) significantly reversed the S1 (10nM, n=5, 24 hours, $p<0.05$) or S1 RBD (10nM, n=4, 24 hours, $p<0.05$) induced increases of TRPV4 sensitive current density (Fig. D−G).

Conclusion: The SARS CoV−2 Spike protein S1 and S1 RBD caused the activation of TRPV4 channels, resulting in increased intracellular Ca2+, which may lead to pulmonary endothelial dysfunction.

Clinical Implications: TRPV4 inhibition appears as a worthy strategy to protect against endothelial dysfunction in Covid−19 patients.


A
CONTROL
S1 1nM
S2 1nM
S1 RBD 1nM
Calcium concentration (nmol/L)
1500
1000
500
0
0 min
10 min
20 min
30 min
12 h
24 h
48 h
Time
* #
B
CONTROL
S1 10nM
S2 10nM
S1 RBD 10nM
C
CONTROL
CONTROL+HC067047
S110nM
S110nM+HC067047
S1RBD10nM
S1RBD10nM+HC067047
ns
**
800
600
400
200
D
Current Density (pA/ pF)
Voltage (mV)
Control
S1
Pretreat with HC067047+S1
E
Current Density (pA/pF)
F
S1 RBD
Pretreat with HC067047+S1 RBD
G