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Title: Regulation of epithelial sodium channel activity by SARS-CoV-1 and SARS-CoV-2 proteins

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Abstract: COVID-19 is caused by SARS-CoV-2 infection, a highly transmissible beta-coronavirus. SARS-CoV-2 produces several different proteins whose roles are not comprehensively understood. First, we tested their ability to form ion channels in the plasma membrane and found that SARS mRNA injection in Xenopus oocytes did not produce functional channels on the plasma membrane. To verify that proteins were being produced, we ran a western blot for the envelope (E) proteins and found good expression in our samples. Since SARS does not produce channels independently, we looked at how they may interact with endogenous ion channels. More specifically, we focused on the epithelial sodium channel (ENaC) and the α3β4 nicotinic acetylcholine channel (nAChR). Both channels are important in the respiratory system, so understanding how the SARS proteins affect them may elucidate how some symptoms develop. Here, we find that both versions of the E protein and SARS-CoV-2 S protein decrease ENaC currents. Currents in α3β4 nAChR are significantly lower in oocytes co-expressing the E proteins and are slightly lower with SARS-CoV-2 S protein. One hypothesis for how the SARS proteins affect membrane protein expression is protein kinase C (PKC) activation. We find that PKC activation with phorbol 12-myristate 13-acetate (PMA) decreases ENaC and the α3β4 activity. We also determined that membrane capacitance decreased following PMA treatment, suggesting PKC activation causes an increase in net endocytosis, which is consistent with prior reports. Further, we find that incubating oocytes in Gö 6976, a PKC inhibitor, did not abolish E or S protein-induced channel inhibition, suggesting that there are additional factors modulated by E and S protein expression. One is the furin-cleavage site on the SARS-CoV-2 S protein, which decreases SARS-CoV-2 S protein-induced ENaC inhibition when mutated. Together, we find that SARS-CoV-1 and SARS-CoV-2 proteins interact with endogenous membrane channels, and these interactions may play a role in disease progression.