The Ca²⁺-activated Cl⁻ channel, TransMEMbrane member 16A (TMEM16A), regulates critical functions including smooth muscle contraction, mucosal secretion, and signal transduction. Despite its importance, we are just beginning to understand TMEM16A’s biochemical and biophysical properties. To study TMEM16A regulation, here we recorded endogenous TMEM16A currents from Xenopus laevis oocytes. Using the inside-out configuration of the patch clamp technique, we found that TMEM16A-conducted currents rundown within seconds of patch excision despite the continued presence of Ca²⁺. Current rundown is common amongst channels regulated by phosphatidylinositol 4,5-bisphosphate (PIP2). Thus, we tested the hypothesis that TMEM16A is potentiated by PIP2 by exposing excited inside-out patches to agents that either increased or sequestered membrane PIP2. We found that following rundown, dioctanoyl-PIP2 (diC8-PIP2) applied with Ca²⁺ recovered TMEM16A-conducted currents by 3.5-fold, but not when applied without Ca²⁺. Conversely, application of dioctanoyl-phosphatidyl inositol (diC8-PI), comprised of the backbone of diC8-PIP2 but lacking the two phosphate groups, had only a nominal effect. We also found that PIP2 sequestering agents, neomycin and anti-PIP2, sped TMEM16A current rundown by 2-fold. Current rundown was slowed by at least 2-fold when we enabled rephosphorylation of PI with Mg-ATP application, or by inhibiting PIP2 dephosphorylation by application of the phosphatase inhibitor β-glycerophosphate. In another series of experiments, we also tested our hypothesis that PIP2 potentiates TMEM16A but within intact cells. We used the two-electrode voltage clamp to record the Ca²⁺-activated Cl⁻ currents from X. laevis oocytes exogenously expressing pseudojanin, a PIP2-depleting enzyme. After the rapamycin-induced dimerization of pseudojanin enabling its translocation to the membrane, we observed that reducing membrane PIP2 also reduced TMEM16A conducted currents in Xenopus laevis oocytes. Taken together, our data demonstrate that PIP2 and Ca²⁺ are both necessary for TMEM16A to pass current.

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