

P004 Adenylyl cyclase type 8 distinguishes between capacitative and OAG-activated Ca^{2+} entry in HEK 293 cells

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TRPC channel family members 3, 6 and 7, which are activated by diacylglycerol (DAG), have been suggested to constitute possible candidates for the elusive capacitative Ca^{2+} entry (CCE) channels. Adenylyl cyclase type 8 (AC8) is activated by CCE in non-excitabile cells, but is not responsive to increases in the cytosolic Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) resulting from release from intracellular stores, ionophore-mediated entry, or arachidonate-activated entry through the plasma membrane. In this study, we exploited this unique dependence of AC8 on CCE to ask whether the DAG analogue, 1-oleyl-2-acetyl-*sn*-glycerol (OAG), activates the same subset of Ca^{2+} channels as store depletion, which triggers CCE. In populations of HEK 293 cells, which endogenously express DAG-activated TRP subunits, OAG evoked a faster and greater influx of Ca^{2+} than CCE. OAG-activated Ca^{2+} entry was additive with CCE but, unlike CCE, it did not stimulate AC8 activity. In single cells, OAG evoked a highly heterogeneous response, whereas CCE occurred as a smooth and sustained rise in $[\text{Ca}^{2+}]_i$. Taken together, our results indicate that, in HEK 293 cells, OAG-activated Ca^{2+} entry is distinct from CCE. The inability of the OAG-activated Ca^{2+} entry pathway to regulate AC8 further reinforces the absolute dependence of this enzyme on CCE.