Pharmacological characterization of novel store-operated calcium entry modulators

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Store-operated calcium entry (SOCE) plays a fundamental role in cellular physiology ranging from differentiation of T-cells to control of muscle function. The exact molecular mechanisms behind this phenomenon have been elusive for a number of years, but it is now thought that the principal components of the machinery are the Ca^{2+}-release-activated-Ca^{2+} (CRAC) channels. CRAC channels are assembled from three fundamental protein complexes: Orai proteins, that form the ion channel pore, the stromal interaction molecule (STIM) proteins, which function as ER calcium sensor and activators of the CRAC channels, and canonical transient receptor potential channels (TRPC). Furthermore, the importance of CRAC channels for human health is underlined by an increasing list of genetic studies that have identified that patients who bear loss-or gain-of-function STIM1/Orai1 mutations suffer from severe health issues, including muscle defects, muscle disturbances, haematopoietic dyscrasias, platelet dysfunction, immunodeficiency, autoimmunity and bleeding disorders. Gain-of-function mutations of STIM1 or Orai1 affect primarily skeletal muscles and platelets, at present three separate disorders (tubular aggregate myopathy (TAM), Stormorken syndrome and York platelet syndrome) are described in the literature and can be reconducted to mutations in one of these two proteins. Based on the structure of two known SOCE inhibitors, pyrazole derivatives Pyr2 and Pyr3, a library of modulators was synthesized and their effects on calcium (Ca^{2+}) entry was investigated in four cellular models, that have different expression of Orai, STIM and TRPC. Starting from 80 compounds, we identified three inhibitors (named AL-1S, AL-1N and NM-3S) which are able to inhibit SOCE with efficiency, similar to Pyr3 on HEK, BV2, HeLa and Jurkat cells. Surprisingly, we identified two potential SOCE activators (named AL-2T and NM-3G), that significantly increase tBHQ-induced Ca^{2+} entry in all three cell models tested. Moreover, our Ca^{2+} signalling data were corroborated in electrophysiological experiments in which both AL-2T and NM-3G significantly potentiated Ca^{2+} currents induced by perfusion of cells with IP_3, a second messenger which triggers Ca^{2+} release from the ER thus activating SOCE. Furthermore, the compounds AL-2T and AL-1S were carried forward for further characterization, performing dose-response curves or viability assays. Promisingly, they are less toxic to cells compared to the parent compound Pyr3. Considering the importance of CRAC channels and their gain-of-function mutations for human health, we have proceeded with mutagenesis to recapitulate the mutations found in the Italian patients affected by TAM (c.322T>A p.F108I, c.343A>T p.I115F and c.326A>G p.H109R for STIM1 and c.734C>T p.P245L and c.290C>G p.S97C for Orai1). Wild type and mutated plasmids were transiently transfected in HEK cells to evaluate SOCE. Given that tubular aggregate myopathy (TAM) is a dominant disorder, it was reasoned that a cellular phenotype should have been observable also in the presence of endogenous wild-type protein. The preliminary experiment performed was the evaluation of our compounds and of Pyr3 on the mutations. We demonstrated that Pyr3 (10 μM) completely abolished SOCE from both wild type and mutated STIM1 or Orai1 over-expressing cells; contrariwise AL-1S (10 μM) had a more moderate effect, leading to a SOCE similar to that observed in control over-expressing cells. It must be acknowledged that this effect is determined by concentration, as when performing concentration-response curves, it was found that higher concentrations (*ie* 100 μM) of AL-1S lead to a complete abolishment of SOCE. In conclusion, we identified novel compounds for modulation of SOCE paving the way towards better understanding of Ca^{2+} entry mechanisms and development of novel therapeutic strategies for pathologies caused by alterations of SOCE.