434 Consequences of pharmacological inhibition of store operated calcium entry on calcium signalling in MDA MB 468 breast cancer cells.

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Introduction. Store-operated calcium entry (SOCE), describes the process whereby there is an influx of calcium ions (Ca^{2+}) after intracellular Ca^{2+} stores are depleted. A remodelling of the molecular components of SOCE is evident in breast cancers of the poor prognosis basal molecular subtype (McAndrew et al, 2011). However, pharmacological studies of this pathway in breast cancer cells have often used non specific SOCE inhibitors, non physiological mechanisms of calcium store depletion and just one basal breast cancer cell line - MDA MB 231 (Yang et al, 2009).
Aims. To assess the effects of the selective SOCE inhibitors YM 58483 and Synta66 on calcium influx mediated by the Ca^{2+} store pump inhibitor cyclopiazonic acid (CPA), the purinergic receptor activator adenosine triphosphate (ATP), the protease-activated receptor-2 (PAR-2) activator trypsin and epidermal growth factor (EGF) in MDA MB 468 basal breast cancer cells in the presence of extracellular Ca^{2+}.
Methods. MDA-MB-468 cells were loaded with the Ca^{2+} sensitive indicator Fluo 4 and cytosolic free Ca^{2+} levels ($[Ca^{2+}]_{cyt}$) were assessed during treatment with CPA, ATP, trypsin and EGF in the absence or presence of YM 58483 or Synta66 using a Fluorescence Imaging Plate Reader (FLIPR).
Results. CPA, ATP, trypsin and EGF exhibited $[Ca^{2+}]_{cyt}$ transients with different degrees of sustained Ca^{2+} influx. The effects of Synta66 and YM-58483 were greatest for CPA and ATP mediated Ca^{2+} influx. Sustained Ca^{2+} influx after stimulation was reduced by 35.1 and 35.5% for CPA (10 μM) and 52.4 and 48.6% for ATP (10 μM) by Synta66 and YM-58483, respectively.
Discussion. These studies define a role for SOCE as a consequence of activation in the regulation of sustained Ca^{2+} influx in MDA-MB-468 basal breast cancer cells.

McAndrew D et al (2011) Mol Cancer Ther. 10:448-60
Yang S et al (2009) Cancer Cell. 15:124-34¶

435 The sweet taste receptor: a novel target for drug discovery?

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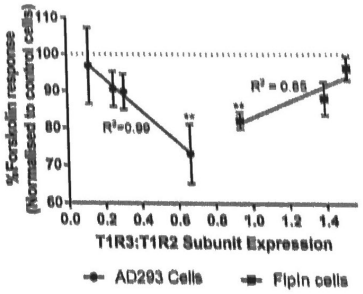

Introduction. Sweet taste receptors are expressed in many tissues throughout the body, and are implicated in obesity and diabetes. The canonical receptor is a heterodimer consisting of subunits T1R2 and T1R3 in a 1:1 ratio. However, in the pancreas and adipose tissue, the expression of these subunits has been shown to be unequal. It is essential to understand if this altered expression profile leads to changes in receptor function, so that this receptor may be harnessed as a novel drug target in the treatment of diabetes and obesity.
Aims. To examine the impact of altering subunit expression on receptor signalling and surface trafficking.
Methods. Heterologous expression systems were generated using either sequentially transfected AD293 cells, or the Flpin system. Subunit expression was quantified by RT-PCR. Signalling through the Gi pathway was measured as a reduction in % forskolin response determined by cAMP assay using the BRET CAMYEL sensor. Surface trafficking was determined by biotinylation pull-down experiments.
Results. Subunit expression closest to 1:1 lead to the greatest functional responses to aspartame, as shown in the figure above. Expression of both sweet taste receptor subunits was found to be predominantly intracellular, and was not improved by 1:1 expression of both subunits.
Discussion. Unequal expression of the two sweet taste receptor subunits lead to an alteration in signalling profile – in this study, a reduction in Gi signalling. This suggests that the sweet taste receptor may either be non-functional, or signals through alternative pathways in tissues where there is unequal expression of subunits. Surprisingly, surface expression did not appear to correlate with functional response. More research is therefore needed to understand tissue-specific signalling profiles, to enable the development of the sweet taste receptor as a novel drug target.