Ophiobolin A is an inhibitor of STIM1/Orai1-mediated current

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Store-operated Ca^{2+} entry (SOCE) plays a critical role in maintaining Ca^{2+} homeostasis and involves the activation of Ca^{2+} influx across the plasma membrane following depletion of Ca^{2+} from the endoplasmic reticulum (Feske, 2009; Putney, 2010). Two proteins, stromal interaction molecule 1 (STIM1) and Orai1 have been recently identified as essential components of Ca^{2+} release activated Ca^{2+} (CRAC) channel, the best characterised mediator of SOCE (Roos *et al.*, 2005; Prakriya *et al.*, 2006). STIM1 predominantly resides in the endoplasmic reticulum (ER) and acts as a Ca^{2+} sensor, while Orai1 has a plasma membrane (PM) localisation and comprises the channel pore. Upon store depletion, STIM1 and Orai1 redistribute in the ER and PM respectively, and co-localise at junctional ER, regions where the ER and PM are juxtaposed. The present study investigates the effects of the fungal toxin ophiobolin A (OphA), a known calmodulin (CaM) inhibitor, on the activation of CRAC current (I_{CRAC}) and distribution of STIM1 and Orai1 in liver cells.

The experiments were conducted on control H4IIE liver cells and H4IIE cells heterologously expressing STIM1 and Orai1. Fura-2 imaging has been used to measure cytoplasmic [Ca^{2+}] and membrane currents were measured by whole cell patch clamping using a computer based patch-clamp amplifier (EPC-9, HEKA Elektronik) and PULSE software (HEKA Elektronik).

It was determined by patch-clamp recordings that OphA inhibited the activation of native I_{CRAC} in control H4-IIE cells stimulated with either inositol 1,4,5-trisphosphate (IP_3) or thapsigargin (TG). Fura-2 imaging revealed that OphA had no detectable effect on intracellular Ca^{2+} release secondary to TG application. However Ca^{2+} entry was entirely inhibited by OphA at a concentration of 20 μM. It is therefore likely that OphA acts *via* a mechanism independent of store depletion. As the biophysical properties of the I_{CRAC} mediated by heterologously expressed STIM1 and Orai1 depend on the relative expression levels of these proteins (Scrimgeour *et al.*, 2009), two transfection rations between STIM1 and Orai1 containing plasmids were used (1:4 and 4:1 of Orai1:STIM1). When STIM1 was co-expressed in excess of Orai1, the resulting current was inhibited almost entirely by OphA. In contrast, the inhibition was reduced when cells were transfected with Orai1 in excess of STIM1. Confocal imaging also revealed differences in the redistribution of STIM1 and Orai1 following application of TG when OphA was present. When STIM1 was expressed alone, its response to TG in the presence of OphA was indistinguishable from control. However, when STIM1 was co-expressed in excess of Orai1, the redistribution of STIM1 was uninhibited whereas Orai1 maintained its contiguous distribution.

The results suggest that OphA is a novel inhibitor of I_{CRAC} and is likely to act by disrupting the interaction between STIM1 and Orai1.

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