Comparison of the mammalian adenylyl cyclase 8 and the *Drosophila* rutabaga adenylyl cyclase in lipid rafts

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Ca^{2+}-stimulated adenylyl cyclases (ACs) play key roles in learning and memory in both mammals and flies. Whereas much attention has focussed on the mammalian Ca^{2+}-stimulated enzymes in terms of their dependence on calmodulin (CaM) regulation in vitro and regulation by Ca^{2+}-entry in vivo, little is known of these aspects for *Drosophila* rutabaga AC, with the exception that *Drosophila* rutabaga AC is also known to be stimulated by Ca^{2+}/CaM. For instance, it is known that the mammalian Ca^{2+}/CaM stimulated adenylyl cyclase 8 (AC8) is required to reside in lipid rafts to be susceptible to the entry of Ca^{2+} (Fagan et al., 2000), but nothing is known of the regulation of the *Drosophila* rutabaga AC by Ca^{2+} nor indeed whether it resides in lipid rafts. Therefore, we asked whether similar behaviour was shown by the *Drosophila* enzyme to the mammalian AC8.

To address this issue, the mammalian AC8, the *Drosophila* rutabaga AC and a truncated form were expressed in HEK293 cells. The *Drosophila* rutabaga AC has a similar overall amino acid sequence to the mammalian AC8, except at the C-terminus, where an additional 1037 amino acids are included. We deleted these extra 1037 amino acids from the C-terminus to produce a truncated form. Cells were fractionated into raft and non-raft fractions via a sucrose gradient after extraction with cold Triton X-100 (about 70% of the total caveolin remained in light fractions; n=3). In further experiments, the lipid rafts were disrupted in all cells by cholesterol depletion using methyl-β-cyclodextrin (about 10% of the total caveolin remained in light fractions; n=3). We find that, like the mammalian enzyme, there is an absolute dependence on Ca^{2+}-entry for the regulating the *Drosophila* rutabaga AC by Ca^{2+}. The *Drosophila* rutabaga AC is also found in lipid rafts along with the lipid raft marker, caveolin, like the mammalian AC8 (80% of the ACs remained in light fractions; n=3). However, the truncated *Drosophila* rutabaga AC is present in both rafts and non-raft fractions (70% of the AC remained in light fractions and 30% in the heavy fractions; n=3). Using cholesterol depletion, the mammalian and *Drosophila* rutabaga is moved out of lipid rafts, whereas the truncated *Drosophila* rutabaga AC persists in rafts and non-raft fractions.

The results lead us to conclude that the *Drosophila* enzyme behaves like the mammalian enzyme unlike the truncated *Drosophila* enzyme. This could mean that the *Drosophila* enzyme might have the same targeting mechanism as the mammalian AC8 and that the C-terminus in the *Drosophila* enzyme might play a key role in this mechanism because the truncated *Drosophila* enzyme does not show the same characteristics in our hands.

To confirm the similar targeting mechanism of the *Drosophila* rutabaga AC and mammalian AC8, ongoing experiments involve caveolae disruption by caveolin-1 knockdown using caveolin-1 shRNA, examination of the consequences of the knockdown on AC fractionation, and measurements of the effect of Ca^{2+}-entry on the AC activity in these caveolin-1 knockdown cells.

K.A. Fagan, K.E. Smith and D.M.F. Cooper (2000) Regulation of the Ca^{2+}-inhibitable adenylyl cyclase type VI by capacitative Ca^{2+}-entry requires localization in cholesterol-rich domains. J. Biol. Chem. **275**, 26530-26537