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Detergent-Free Solubilisation of the Adenosine 2a Receptor and the Calcitonin Receptor Using Styrene Maleic Acid Lipid Particles (SMALPs)

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The ability to produce detergent-solubilised preparations of G-protein-coupled receptors (GPCRs) has been exploited by many groups to increase our understanding of receptor mechanisms and for use in drug discovery assays, despite the universal acknowledgement that exposing GPCRs to detergents perturbs and destabilises the receptor. In a separate study, we have utilised styrene maleic acid (SMA) lipid particles (SMALPs) to purify the adenosine 2a receptor (A2aR) from yeast membranes without the use of detergent at any stage (1). Here we establish that our SMALP-solubilsation can be used to generate nano-scale particles of mammalian cell membrane containing GPCR (family A or family B).

HA-tagged A2aR was transiently expressed in HEK 293T cells; HA-tagged calcitonin receptor (CTR) was transiently expressed in COS-7 cells in the presence or absence of myc-tagged receptor activity modifying protein 1 (RAMP-1). Cells were incubated with SMA (2.0 % w/v final concentration), 5 IU/ml benzonase and complete EDTA-free protease inhibitor for 1 h at 37 ^{o}C, followed by 100,000 x g centrifugation for 1 h at 4 ^{o}C. The supernatant contained the GPCR-SMALP. Radioligand binding assays were performed with [^{3}H]ZM241385 or [^{125}I]salmon calcitonin ([^{125}I]sCT) as tracer for A2aR and CTR respectively, with bound and free ligand separated using Sephadex spin columns.

The A2aR-SMALP bound ZM241385 with a pKi of 8.53 ± 0.04, compared to 8.87 ± 0.1 in HEK 293T cell membranes (n=3). Likewise, the pKi values (n=3) for the ligands NECA (SMALP, 5.39 ± 0.41; membranes, 5.29 ± 0.1) and theophylline (SMALP, 4.97 ± 0.27; membranes, 4.87 ± 0.12) were similar. The A2aR-SMALP also possessed increased thermostability compared to the corresponding detergent (dodecyl-D-maltopyranoside; DDM)-solubilised A2aR with t½ values (n=3) at 37 ^{o}C of 148 ± 13 min and 21 ± 7 min for A2aR-SMALP and A2aR-DDM respectively. The A2aR-SMALP was stable when stored at 4 ^{o}C and was also resistant to repeated freeze-thaw cycles.

The ability of SMALP to support specific binding was not restricted to family A GPCRs, as specific binding of [^{125}I]sCT was also observed with the family B receptor CTR in a SMALP.

In summary, the spontaneous encapsulation of GPCRs, surrounded by native lipid, into a nano-scale membrane disc stabilised by SMA polymer presents new opportunities for studying GPCRs free from the detrimental effects of detergent.

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1. Jamshad, M *et al.* (2015) *Biosci. Rep.* (in press)