Solubilisation of CGRP Receptors by Styrene Maleic Acid Lipid Particles (SMALPs) from transfected cells and native tissues

Membrane proteins are normally solubilised with detergents but these molecules may strip away native lipids, perturbing the properties of the protein. We have recently reported the solubilisation and purification of the adenosine 2a receptor, a family A G protein coupled receptor (GPCR) using styrene maleic acid (SMA) co-polymers. These remove the protein with its associated lipids in a nanoparticle, a SMA lipid particle (SMALP) (1). Here we investigate the use of SMA to solubilise the receptor for calcitonin gene-related peptide (CGRP), a heterodimer between calcitonin receptor-like receptor (CLR), a family B GPCR and the accessory protein, receptor activity modifying protein 1 (RAMP1) (2).

Cos 7 cells were co-transfected in 10 cm dishes with haemagglutinin-tagged human CLR and myc-tagged RAMP1 (2). Membranes were prepared as previously described (2). For labelling, they were incubated with 0.5 nM of human ^{125}I-CGRP (Perkin Elmer) for 30 min at room temperature (with or without 1 μM unlabelled CGRP to define non-specific binding) in 2mM $MgCl_2$, 20 mM Hepes pH 7.4. Membranes were pelleted and resuspended at 1mg/ml in SMA co-polymer (2.5 % w/v final concentration in 50mM NaCl, 12.5% glycerol, 500mM Tris, pH 8) for 1 h at 25^{o} C. The undissolved membranes were pelleted and radioligand binding was performed on the GPCR in the supernatant with spin columns as described previously (1). Membranes from rat spleen and cerebellum were prepared as described previously (3) and then labelled and solubilised as for Cos 7 cells.

Cos 7 cell membranes expressing CGRP receptors were labelled with ^{125}I-CGRP before solubilisation as this ligand binds to SMA, precluding labelling after solubilisation. Following SMALP extraction for 1 h at 25^{o} C, 64 ± 8% (n=5) of the ^{125}I-CGRP specifically bound to the membranes was recovered in the soluble fraction, with 14 ± 1% of the specifically bound ligand left in the post-solubilisation pellet. There was no advantage to a 24 h solubilisation at 4 ^{o} C (supernatant recovery 49 ± 22%, n=3). As CGRP is an agonist, the majority of the receptor to which it binds would be predicted to be coupled to a G protein and addition of 100 μM GppNHp reduced specific binding by 60 ± 8% (n=3). Spleen and cerebellum both express high numbers of CGRP binding sites (3); after SMA extraction, 49 ± 10% and 40 ± 13% (n=3) of specifically bound ^{125}I-CGRP from the two respective tissues was solubilised.

These experiments show that it is possible to use SMA to solubilise a family B GPCR in complex with a RAMP. As RAMPs frequently associate with these GPCRs to modify their properties (2), this is an important finding. Furthermore, the SMALPs isolate the receptor with a G protein, as evidenced by the sensitivity of the ^{125}I-CGRP binding to GppNHp, a GTP analogue which will dissociate the G protein (3). This is also the first demonstration of the use of SMALPs to isolate GPCRs from native tissues, allowing the study of receptors from animal models.

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2) Barwell, J *et al.* (2011). *Biochim. Biophys. Acta*. **1813**:1906–16.
3) van Rossum, D *et al.* (1993) *Brain Res.* **617**:249–57.