

Antagonist-dependant changes in the conformation of a styrene maleic acid copolymer (SMALP)-solubilised adenosine 2a receptor labelled with IAEDANS

Membrane proteins are normally solubilised with detergents, but these perturb the properties of the protein. To overcome this, we have previously reported the solubilisation of the adenosine 2a receptor (A2aR) using (SMALPs) [1]. We have also reported that, using endogenous tryptophan fluorescence, the antagonist ZM241385 produced a concentration-dependent change in emission, suggesting that the receptor is stabilised in a form distinct from the inactive ground state [2]. To further investigate this, we have labelled the receptor with the fluorescent reporter IAEDANS.

An N-terminal His-tagged A2aR with the mutations A231C and I106W was purified from *Pichia pastoris* as described previously [1, 2]. The receptor was labelled with IAEDANS and fluorescence intensity was recorded with excitation at 340 nm [3]. The receptor was challenged with increasing concentrations of the agonist NECA and the antagonist ZM241385. Concentration-response curves were fitted to give E_{max} , minimum and pEC_{50}

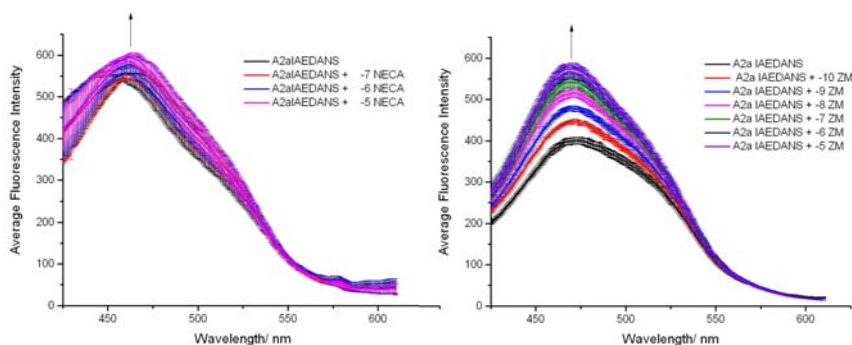


Fig 1. Fluorescence emission spectra of the IAEDANS-labelled A2aR challenged with NECA (left) and ZM241385 (right). Values are means \pm sem of three independent experiments.

ZM241385 caused a large increase in fluorescence intensity, with a pEC_{50} of 8.74 ± 0.32 ($n=3$). By contrast, NECA was caused a much smaller change. This is similar to our previous results using endogenous tryptophan fluorescence [2], although it was substantially less potent in those studies (pEC_{50} 7.00 ± 0.10 , $n=3$). This gives further evidence that SMALPs stabilise the A2aR in a form that is closer to the agonist-stabilised active state rather than a fully inactive receptor. This work was supported by BBSRC grants BB/1019960 and BB/1020349.

References

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3. <https://tools.thermofisher.com/content/sfs/manuals/mp00003.pdf1>