Using the fluorescent antagonist, XAC-X-BY630 to quantify antagonist-adenosine A_3 receptor complexes in membrane microdomains of single living cells.

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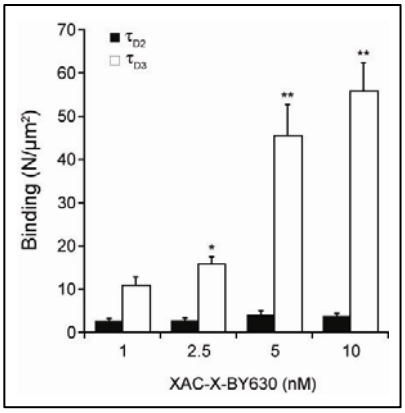
The adenosine A_3 (A_3-AR) receptor is one of four known G-protein coupled receptors activated by the nucleoside adenosine. We have previously demonstrated the heterogeneity of agonist-A_3-AR complexes in the membrane of Chinese Hamster Ovary (CHO-A_3) cells using fluorescence correlation spectroscopy (FCS) (Cordeaux *et al.*, 2008). Here, we describe the characterisation of XAC-X-BY630 (Briddon *et al.*, 2004), as a fluorescent antagonist label for the A_3-AR and use it to demonstrate a similar heterogeneity in antagonist-A_3-AR complexes in CHO-A_3 cells.

Confocal imaging and FCS were carried out essentially as described (Cordeaux *et al.*, 2004). Confocal imaging showed clear membrane labelling of CHO cells expressing a YFP-tagged version of the A_3-AR following incubation with XAC-X-BY630 (25nM, 10min, 22°C), which was substantially reduced by MRS1220 (100nM, 30min, 37°C) (n=3). Initial FCS experiments using CHO-A_3 cells incubated with XAC-X-BY630 (1-10nM, 10min, 22°C) revealed both fast- (τ_{D2}) and slow-moving (τ_{D3}) complexes at the cell membrane, with average diffusion co-efficients of 1.58±0.16 $\mu m^2/s$ and 0.081±0.007 $\mu m^2/s$, respectively (mean±s.e.mean, n=100). At concentrations of XAC-X-BY630 ranging from 1-10nM the amount of τ_{D3}, but not τ_{D2}, increased in a concentration-dependent manner (Fig.1 P<.0001, one-way ANOVA). However, both species represent antagonist-receptor complexes, since the binding of both τ_{D2} and τ_{D3} following addition of 2.5 and 5nM XAC-X-BY630 was significantly reduced following pre-incubation of cells with the specific A_3-AR antagonist MRS1220 (300nM, 30min, 37°C) (P<0.01, t-test, n=12).

Fig.1 Quantification of XAC-X-BY630 binding in CHO-A_3 cells using FCS. CHO-A_3 cells were incubated with XAC-X-BY630 (10min, 22°C) and levels of τ_{D2} and τ_{D3} determined. mean±s.e.mean, n=34-48. *P<0.05, **P<0.001 vs 1nM, Newman-Keuls test.



These studies show, therefore, that XAC-X-BY630 is an A_3-AR antagonist suitable for use in FCS. In addition, as with agonist-A_3-AR complexes, antagonist-A_3-AR complexes exist in both fast- and slow-moving forms in the cell membrane.

Cordeaux, Y *et al.* (2008) *FASEB. J.,* **22**, 850.
Briddon, S et al. (2004) Proc. Natl. Acad. Sci. USA., **101**, 4673.

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