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The binding kinetics of a fluorescent agonist at the human adenosine A_3 receptor in whole cells

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The synthesis of ABA-X-BY630, a novel N^6-aminoalkyl derivative of adenosine which incorporates the BODIPY [630/650] fluorophore has been described previously (Middleton *et al*, 2007). This study has investigated the association and dissociation kinetics of ABA-X-BY630 at the adenosine A_3 receptors in the absence and presence of an unlabelled antagonist at a single cell level.

CHO cells stably expressing the human adenosine A_3 receptor were exposed to HEPES-buffered saline solution (HBSS) containing 100 nM ABA-X-BY630 for approximately 70 seconds after which time cells were washed with HBSS alone or in the presence of 1 μM MRS 1220. A perfusion system with a flow rate of approximately 20 $mL.min^{-1}$ was used for the addition and removal of ligand. For the duration of the experiment, confocal fluorescence and phase images were obtained at one second intervals using a Zeiss 510 confocal microscope. Each replicate represents the average fluorescence intensity (%) of the plasma membranes of 10 cells.

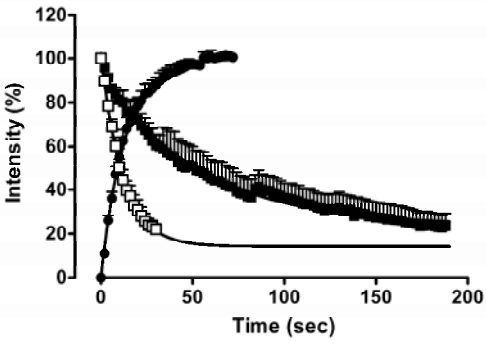

Figure 1: ABA-BY630 association (●) and dissociation kinetics in the absence (■) and presence (□) of 1μM MRS 1220. Data points represent the mean ± S.E.M. of 40-70 cells from 4-7 separate experiments. Two second intervals shown for clarity.

The association of ABA-X-BY630 at the adenosine A_3 receptor was monophasic with an association rate constant, k_{on}, of 574700 ± 19000 $M^{-1}sec^{-1}$ (mean ± S.E.M. n=7). ABA-X-BY630 dissociation was determined under conditions reflecting that of infinite dilution in the absence and presence of the selective adenosine A_3 antagonist, MRS 1220. Under both conditions, ABA-X-BY630 dissociation was monophasic, however the dissociation rate in the absence of antagonist (k_{off} = 0.019 ± 0.001 sec^{-1}, mean ± S.E.M., n=4) was significantly slower than that in the presence of 1 μM MRS 1220 (k_{off} = 0.080 ± 0.007 sec^{-1}, mean ± S.E.M., n=4).

In summary, confocal imaging has been used to directly measure, at single cell level, the binding kinetics of the fluorescent adenosine agonist, ABA-X-BY630. In addition, the perfusion system allows for the rapid removal of ligand and as such the comparison of ABA-X-BY630 dissociation in the absence and presence of antagonist. Under infinite dilution conditions, the dissociate rate of ABA-X-BY630 should be unaffected by the presence of a simple competitive antagonist. Therefore the ability of MRS 1220 to enhance the dissociation rate of ABA-X-BY630 suggests that there may be a negatively cooperative interaction occurring between the two ligands.

Middleton, R.J. *et al*. (2007) *J. Med. Chem.*, 50, 782-793