Kinetic analysis of the binding of xanthine amine congener and a fluorescent analogue (XAC-X-BY630) at the human adenosine A_1 receptor using a live cell radioligand binding assay

Jonathan Hern, Stephen Briddon, Stephen Hill. The University of Nottingham, Nottingham, United Kingdom.

Single cell pharmacology techniques such as fluorescence correlation spectroscopy (FCS) require fluorescent small molecule ligands for the receptor of interest (Briddon & Hill, 2007). However, the pharmacology of these ligands requires careful characterisation. Here we quantitatively compare the kinetics of binding at the human adenosine A_1 receptor (A_1-AR) of the antagonist XAC and its fluorescent derivative, XAC-X-BY630 (Briddon *et al.*, 2004) under the same conditions used for live cell imaging and FCS.

Rate equations derived from the law of mass action can describe the association and dissociation rate constants of a ligand by means of its effect on the binding kinetics of an appropriate competing radioligand (Motulsky & Mahan, 1984). Here, a live cell microwell plate assay was used to determine the association and dissociation rate constants of XAC and XAC-X-BY630 binding in the presence of the A_1-AR antagonist [^{3}H]DPCPX. Results were analysed by simultaneous non-linear least squares analysis of [^{3}H]DPCPX association, dissociation and competition binding curves using the equations derived by Motulsky and Mahan (1984). [^{3}H]DPCPX dissociation was achieved by a two-fold volume dilution containing an excess concentration of DPCPX. The binding of [^{3}H]DPCPX at a CHO cell line stably expressing A_1-AR (B_{max} = 263±26 fmol/mg protein, n=3) was consistent with a simple reversible bimolecular interaction (K_{on} = 4.7±0.5 x10^7 M^{-1}.min^{-1}; K_{off} = 0.062±0.005 min^{-1}, n=10) and the calculated log K_d value (-8.87±0.03, n=10) was in agreement with that from [^{3}H]DPCPX saturation equilibrium binding experiments (-8.84±0.11, n=3).

XAC-X-BY630 was previously shown to exhibit ten-fold lower affinity and potency than XAC at the A_1 receptor in an equilibrium whole cell binding assay (Briddon *et al.*, 2004). In the present study the log dissociation constant of XAC was also ten-fold higher than XAC-X-BY630 when determined by competition kinetics (log K_d -6.65±0.02 (n=6) and -5.76±0.22 (n=4) XAC and XAC-X-BY630 respectively). This difference in K_d between XAC and XAC-X-BY630 was manifest as a difference in k_{off} (0.13±0.03 & 0.29±0.11 min^{-1} respectively) but predominantly a change in k_{on} (5.8±0.9 x10^5 & 1.4±0.3 x10^5 M^{-1}.min^{-1} respectively).

These results show that whole cell radioligand binding assays can be used to measure the kinetics of binding of both radiolabelled and unlabelled antagonists at the human A_1-AR.

Briddon SJ *et al.* (2004) *Proc. Natl. Acad. Sci. (USA)* **101**, 4673.
Briddon SJ & Hill SJ (2007) *Trends in Pharmacological Sciences* **28**, 637.
Motulsky HJ & Mahan LC (1984) *Mol. Pharmacol.* **25**, 1.

This work was supported by BBSRC grant number BBD5215811.