Proceedings of the British Pharmacological Society at http://www.pA2online.org/abstracts/Vol19Issue1abst026P.pdf

Influence of the kinetic profile of labelled ligands on measured binding rates of unlabelled antagonists at the adenosine A_3 receptor.

M. Bouzo-Lorenzo[1], L. Stoddart[1], L. Xia[2], A. IJzerman[2], L. Heitman[2], S. Briddon[1], S. Hill[1]. [1]COMPARE & Division of Physiology, Pharmacology and Neuroscience, School of Life Science, Nottingham, United Kingdom, [2]Division of Medicinal Chemistry, Leiden Academic Centre for Drug Research, Leiden, Netherlands.

Introduction It is becoming increasing clear that compounds that bind to the same G protein-coupled receptor (GPCR) can have markedly different rates of association and dissociation which can influence clinical efficacy (1). For GPCRs, binding rates are often measured indirectly by monitoring the effect an unlabelled compound on the association of labelled ligand. Ligands can be labelled with radioactive isotopes and used in radioligand binding assays or with flurophores to be used in resonance energy transfer techniques such as bioluminescence resonance energy transfer (BRET) (2). A range of fluorescent ligands have been described for the adenosine A_3 receptor (A_3R) (3) and in this study we evaluated the influence of the kinetic profile of labelled ligands on measured binding rates of unlabelled antagonists.

Methods Equilibrium NanoBRET ligand binding assays on HEK293 cells stably expressing nanoluciferase (Nluc) tagged A_3R (NL-A_3R) were performed as previously reported (2). For kinetic studies, cells were incubated with 10 µM furimazine for 10 min prior the addition of the fluorescent ligand in the presence or absence of unlabelled ligands. Radioligand binding assays were performed on membrane preparations from HEK293 cells expressing NL-A_3R. Equilibrium and kinetic assays were performed as described previously (4).

Results The kinetic profile of three labelled ligands, the *S*,*S* stereoisomer XAC-*S*-ser-*S*-tyr-X-BY630, pyrazolol-BY630 and [^{3}H]PSB11, are shown in Table 1. XAC-ser-tyr-X-BY630 was found to have the longest residence time at A_3R. The association and dissociation rates of three antagonists PSB-11, GIFT330 and LUF7565 were also determined using either XAC-ser-tyr-X-BY630, pyrazolol-BY630 as the labelled probe. The kinetic profiles of GIFT330 and LUF7565 were also measured using [^{3}H]PSB-11. There was close agreement in the values of measured with pyrzolol-BY630 and [^{3}H]PSB-11 but there was little similarity to those obtained using XAC-*S*-ser-*S*-tyr-X-BY630 indicating that it is difficult to determine the kinetic parameters of unlabelled ligands using a ligand with a very long residence time.

	pK_D	K_{on} (x10^6, M^{-1} s^{-1})	K_{off} (min^{-1})	Residence time (min)	*n*
XAC-ser-tyr-X-BY630	8.57 ± 0.15	1.66 ± 0.36	0.0043±0.0009	288 ± 62	5
Pyrazolo-BY630	7.35 ± 0.10	3.67 ± 0.62	0.15±0.02	6.8 ± 0.8	4
[^{3}H]-PSB11	8.04 ± 0.08	2.75 ± 0.52	0.023±0.002	44.6 ± 3.9	5

Table 1 Kinetic parameters of XAC-ser-tyr-X-BY630, pyrazolol-BY630 and [^{3}H]PSB11 measured at NL-A_3R Data represent mean±SEM of n experiments

Conclusions. The data shown here indicates that care needs to be taken in selecting the appropriate labelled ligand as probes with slow dissociation rates can make it problematic to determine the kinetics of unlabelled ligands.

References

(1) Schuetz et al (2017) Drug Discov Today 22: 896-911.
(2) Stoddart et al (2015) Mat Methods 12:611-633.
(3) Kozma et al (2013) Bioorg Med Chem Lett 23: 26-36.
(4) Xia et al (2018) Biochem Pharmacol Epub ahead of print (https://doi.org/10.1016/j.bcp.2017.12.026)