CHARACTERIZATION OF A FLUORESCENT AGONIST ACTING AT THE HUMAN ADENOSINE-A3 RECEPTOR IN CHO CELLS

Y. Cordeaux[†], R.J. Middleton[‡], B. Kellam[‡] & S.J. Hill[†], [†]Institute of Cell Signalling, School of Biomedical Sciences, University of Nottingham, NG7 2UH. And [‡]Centre for Biomolecular Sciences, University Park, Nottingham, NG7 2RD, U.K.

We have previously described the synthesis of the ligand ABEA-X-BY630; a fluorescent derivative of the non-selective adenosine agonist NECA (Briddon *et al*, 2002) and shown that it acts as an agonist at both the human adenosine-A_1 and -A_{2B} receptors (Briddon *et al*, 2002, Cordeaux *et al*. 2003). In the present study we have investigated the ability of this ligand to activate the Gi-coupled adenosine-A_3 receptor and to modulate cAMP accumulation, inositol phosphate production and calcium signalling.

Human adenosine-A_3 receptor cDNA (UMR cDNA Resource center, USA) was subcloned into the vector pcDNA3.1 and transfected into CHO cells by lipofectamine™. Stable transfects ($CHOA_3$ cells) were selected in the presence of 500μg/ml geneticin and clones identified by dilution cloning. $CHOA_3$ cells were cultured and assays performed (to measure the accumulation of [^{3}H]cAMP or [^{3}H]inositol phosphates) as previously described (Cordeaux *et al*. 2000). For live cell confocal imaging, cells were grown to 70% confluency in 35mm glass-bottomed MatTek™ dishes and loaded with Fluo 4AM (2.3μM, 1hr, 37°C). Cells were washed and incubated at 37°C in HEPES-buffered saline for 30mins prior to imaging. Images were obtained using a Zeiss LSM510 confocal microscope, using simultaneous excitation at 633nm and 488nm and collected every 4 seconds for 30mins after addition of ABEA-X-BY630 (100nM). Data are expressed as mean ± s.e.m.

In $CHOA_3$ cells, NECA, IB-MECA (an A_3-selective agonist) and ABEA-X-BY630 produced a concentration-dependent inhibition of forskolin-stimulated [^{3}H]cAMP production (pEC_{50}; 8.34 ±0.13, n=4, 8.89±0.12, n=3 and 8.88±0.18, n=3 respectively). The extents to which they inhibited forskolin-stimulated [^{3}H]cAMP production (96±2%, 88±3% and 96±2% respectively) were similar. For each ligand the dose response could be shifted to the right by pre-incubating the cells with the A_3-selective antagonist MRS1220 (pK_Bs; 9.47±0.07, n=4, 9.39±0.08, n=3 and 9.44±0.10, n=3 respectively). The ligands also caused concentration-dependent increases in [^{3}H]inositol phosphate production, (pEC_{50}; 7.00±0.07, n=3, 8.09±0.02, n=3 and 7.03±0.18, n=3 respectively), with similar maximal responses (2.67±0.07, 2.86±0.06 and 2.39±0.31-fold of basal response).

By confocal imaging, ABEA-X-BY630 was shown to label the plasma membrane of these cells and cause a subsequent increase in intracellular calcium. Pre-incubation of the cells with MRS1220 (1μM, 30mins) reduced membrane labeling and reduced the number of cells showing an increase in calcium (from 72 ± 3% to 24 ± 6%, n=3; each approximately 30 cells).

In summary, ABEA-X-BY630 is an agonist at the human adenosine-A_3 receptor which may prove a useful tool for studying this receptor in both healthy and diseased human tissue.

Briddon, S.J. *et al.* (2002) *Br. J. Pharmacol.*, **138**, 126P.
Cordeaux, Y. *et al.* (2000) *Mol. Pharm.*, **58**, 1075.
Cordeaux, Y. *et al.* (2003) *Br. J. Pharmacol.*, **140**, 16P.

We thank the Wellcome Trust for financial support (ref 066817) and Diane Hall for technical assistance.