

016P ABEA-BY630 IS A FLUORESCENT AGONIST AT A_{2B} ADENOSINE RECEPTORS IN HUMAN PROSTATIC STROMAL CELLS

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We have previously described the synthesis of ABEA-BY630, a fluorescent derivative of the adenosine agonist NECA (5'-N-ethylcarboxyamidoadenosine) and described its agonist activity at Gi/o-coupled human A₁ adenosine receptors expressed in CHO cells (Bridson *et al.* 2003). The aim of the present study was to determine whether the same ligand was able to stimulate Gs-coupled A₂ adenosine receptors that are endogenously expressed in human prostatic stromal cells.

Human prostatic stromal cells were isolated from benign prostatic tissue and cultured as previously described (Abdul-Hamid *et al.* 2001). Cells were grown from one cell line and functional assays performed on cells of passage numbers 4-8. Cells were grown in 24-well cluster dishes and [³H]cAMP accumulation measured as described previously (Cordeaux *et al.* 2000). Where stated, cells were pre-incubated with the antagonists; ZM241385, XAC or SCH58261 (100nM), 15 minutes prior to incubation with agonist.

In human prostatic stromal cells, the agonist NECA stimulated [³H]cAMP accumulation in a concentration-dependent manner (pEC₅₀ = 5.66 ± 0.04, mean ± s.e. mean, n=19). Pre-incubation of cells with the non-selective antagonist XAC caused a rightward shift in the concentration-response curve to NECA

(pK_B = 7.85 ± 0.08, n=3). The weakly A_{2A}-selective antagonist ZM241385 produced a similar shift (pK_B = 7.72 ± 0.04, n=3). The highly selective A_{2A} antagonist SCH58261 (100nM), however, did not alter the NECA response (pEC₅₀; 5.27 ± 0.05 and 5.59 ± 0.17, with and without SCH58261, respectively, n=3). Moreover, the A_{2A}-selective agonist CGS21680 only produced a small [³H]cAMP response (10 ± 4% of NECA maximum at 0.1mM) in these cells at a concentration above 10µM (n=3). Hence, the effects of NECA in these cells can be attributed to activation of the A_{2B} adenosine receptor.

The fluorescent ligand ABEA-BY630 also stimulated [³H]cAMP accumulation in these cells with similar potency to that of NECA (pEC₅₀ = 6.32 ± 0.08, n=4). This stimulation was completely inhibited by preincubating the cells with XAC (1µM, 30 minutes, n=3). Relative to NECA, however, ABEA-BY630 was a partial agonist (37 ± 5 % of NECA maximum, n=4). This agonist may therefore be a useful tool for visualising adenosine receptors in primary cells, and studying their function using fluorescence-based techniques.

Abdul-Hamid MA *et al.* (2001) Br. J.Pharmacol 134: 173P

Bridson SJ *et al.* (2003) Br. J.Pharmacol. (Brighton BPS meeting)

Cordeaux Y *et al.* (2000) Mol. Pharmacol. 58 (5), 1075-1084

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