FUNCTIONAL PROPERTIES OF FLUORESCENT AGONISTS AT THE HUMAN ADENOSINE-A_1 AND -A_3 RECEPTORS

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The synthesis of novel N^6-aminoalkyl derivatives of adenosine or adenosine 5'-*N*-ethyl carboxamide (NECA) which incorporate the BODIPY [630/650] fluorophore has been described previously (Middleton *et al*, 2007). This study has investigated the ability of these ligands to mediate an increase in intracellular calcium through the stimulation of either the human adenosine-A_1 or -A_3 receptors.

Intracellular calcium mobilisation was measured using a fluorescence plate reader (FLEXstation, Molecular Devices). CHO cells (expressing either the human A_1 or A_3 receptors) were grown to confluency in 96-well black-walled plates and incubated in 100 μL HEPES-buffered saline containing 0.1% BSA, 2.5 mM probenecid, 0.5 mM brilliant black, 2.3 μM Fluo 4AM and 0.023% pluronic acid at 37°C for 1 hour. Fluorescence was measured for 200 seconds with the addition of 20 μL HEPES-buffered saline in the absence or presence of agonist at 15 seconds.

At both the adenosine-A_1 and -A_3 receptors, each fluorescent ligand stimulated concentration-dependent increases in intracellular calcium. All of the fluorescent ligands were partial agonists at the adenosine-A_1 receptor, as the maximal response in each case was significantly less then that of the full agonist, NECA. In contrast, at the adenosine-A_3 receptor only ABA-X-BY630 mediated a response that was significantly less then that of NECA, with ABEA-X-BY630, APEA-X-630, AoEA-X-BY630 and APrEA-X-BY630 all acting as full agonists (Table 1).

Table 1: The potency ($LogEC_{50}$) and maximal effect (E_{MAX}, % of NECA maximium) of the fluorescent adenosine receptor agonists. Values are mean ± S.E.M. of 3-4 experiments conducted in triplicate.

		ABA-X-BY630	ABEA-X-BY630	APEA-X-BY630	AoEA-X-BY630	APrEA-X-BY630
$CHOA_1$	$LogEC_{50}$	-6.22 ± 0.11	-6.16 ± 0.22	-6.23 ± 0.06	-6.14 ± 0.07	-5.99 ± 0.07
	E_{MAX}	52.5 ± 2.9*	45.1 ± 4.6*	64.7 ± 5.7*	45.4 ± 5.6*	63.3 ± 4.3*
$CHOA_3$	$LogEC_{50}$	-6.15 ± 0.02	-6.49 ± 0.09	-6.57 ± 0.05	-6.77 ± 0.11	-6.63 ± 0.11
	E_{MAX}	32.5 ± 0.8*	99.7 ± 14.1	100.7 ± 7.6	103.2 ± 7.5	106.6 ± 12.4

*significantly different ($p<0.05$; one way ANOVA, post-hoc Dunnett's test) from the corresponding NECA value. ABA-X-BY630, ABEA-X-BY630, APEA-X-BY630, AoEA-X-BY630 and APrEA-X-BY630 correspond to the compounds 14, 9b, 9c, 9d and 9a respectively in Middleton *et al* (2007).

In summary, although each of the N^6-aminoalkyl derivatives of adenosine or NECA are able to mediate an increase in intracellular calcium, the agonist profile, in particular the efficacy, of these ligands is markedly different at the human adenosine-A_1 and -A_3 receptors.

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