Characterising signaling bias of novel Adenosine A_1 receptor agonists

The adenosine receptors (ARs) are family A G protein-coupled receptors (GPCRs) that exist as 4 subtypes; A_1, A_{2A}, A_{2B} and A_3, each with ~50-60% homology [1]. All four ARs modulate cAMP levels, with the A_1R and A_3R coupling to $G\alpha_i$ reducing cellular cAMP, while the A_2Rs stimulate cAMP production via $G\alpha_s$ activation. All ARs are activated by the purinergic nucleotide, adenosine, and 2 synthetic adenosine derivatives; 5'-*N*-ethylcarboxamidoadenosine (NECA) and chloro-N^6-cyclopentyladenosine (CCPA). We have developed novel AR ligands, some of which display A_1R selectivity [2]. In an attempt to investigate which $G\alpha_{i/o}$ proteins couple to the A_1R we used pertussis toxin (PTX), to inhibit the action of these, at the A_1R. CHO cells, stably expressing the A_1R (CHO-A_1R), treated in this manner, were subsequently stimulated with our ligands, and cAMP levels measured. Interestingly we observed cAMP production upon PTX treatment (Table 1.), indicative of a $G\alpha_s$ component to the A_1Rs repertoire. Whilst this has been observed before [3] it has not been seen to such significant levels, and has not been quantified. Thus here we present a quantification of the signaling bias between the canonical, $G\alpha_i$, pathway, and non-canonical $G\alpha_s$ (Figure 1.). It is interesting to note that the ligand that displays the most extensive $G\alpha_i$ is the natural cognate ligand, adenosine. These agonists are currently the focus of on-going studies pertaining to; calcium mobilisation, ERK activation, and which inhibitory G proteins they activate.

Table 1. Potency and E_{max} for CHO-A_1R cells

Ligand	$G\alpha_i$ [2]		PTX ($G\alpha_s$)	
	pEC_{50}	E_{max}[a]	pEC_{50}	E_{max}
NECA	-9.7 ±0.2	59 ±3.9	-6.7 ±0.2****	44 ±2.8*
Adenosine	-9.7 ±0.3	59 ±5.2	-4.7 ±0.2****	58 ±4.9
CCPA	-8.8 ±0.2	69 ±4.3	-7.0 ±0.3****	38 ±3.6****
cmpd 5	-6.9 ±0.2	69 ±4.3	-5.5 ±0.3**	33 ±5.3****
cmpd 6	-9.2 ±0.2	56 ±3.2	-6.7 ±0.5****	26 ±4.0****
cmpd 16	-7.7 ±0.3	41 ±4.8	-6.2 ±0.3***	37 ±4.1
cmpd 18	-8.7 ±0.4	39 ±4.7	-7.1 ±0.3***	22 ±2.8*
cmpd 20	-10.5 ±0.3	48 ±3.6	-7.1 ± 0.2****	40 ±3.9
cmpd 21	-8.2 ±0.2	47 ±4.7	-6.7 ±0.2**	47 ±4.0

[a] – E_{max} for $G\alpha_i$ coupling calculated as percentage inhibition when stimulated with 10μM forskolin.
Statistical significance to $G\alpha_i$ calculated using a one-way ANOVA with Dunnet's post-test (*, p<0.05, **, p<0.01, ***, p<0.001, ****, p<0.0001)

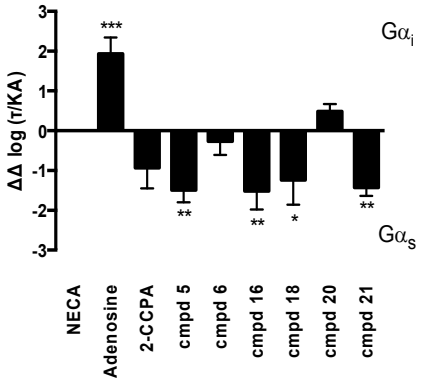

Figure 1. Relative bias for the A_1R. Signalling bias was calculated relative to NECA as ΔΔ log(τ/KA) [4]. Statistical difference to NECA was determined using a one-way ANOVA with a Bonferroni's post-test (*, p < 0.05, **, p < 0.01, ***, p ,0.001). Data are the mean of 5 individual data sets ± SEM.

[1] Jacobson and Gao (2006) *Nat Rev Drug Discov* **5**:247–264,
[2] Knight *et al* (2016) *J Med Chem* **59**:947-964,
[3] Baker and Hill (2007) *J Pharmacol Exp Ther* **320**:218–282,
[4] Baltos *et al* (2016) *Biochem Pharmacol* **99**:101–112.
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