THE EFFECT OF BRILLIANT BLACK BN ON AGONIST ANTAGONIST INTERACTIONS AT THE HUMAN ADENOSINE-A_1 AND -A_3 RECEPTORS

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The synthesis of XAC-BY630, a novel xanthine amine congener (XAC) derivative which incorporates the BODIPY [630/650] fluorophore has been described previously (Briddon *et al*, 2004). Brilliant Black BN is commonly used in experiments involving calcium-sensitive fluorescent dyes (e.g. Fluo 4AM) to reduce the signal from extracellular dye (Roth et al., 2006). This study investigated the effect of Brilliant Black BN on the ability of the agonist 5'-*N*-ethyl carboxamide (NECA) and the antagonists XAC and XAC-BY630 to bind to the human adenosine-A_1 and -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 at 37°C for 45 minutes in 100 µL DMEM containing 10% FCS, 2.5 mM probenecid, 2.3 µM Fluo 4AM and 0.023% pluronic acid. Cells were then washed twice with PBS and incubated in 100 µL HEPES-buffered saline containing 2.5 mM probenecid in the presence or absence of antagonist and/or Brilliant Black BN at 37°C for 30 minutes. Fluorescence was then measured in the presence of increasing concentrations of NECA. At both the adenosine-A_1 and -A_3 receptors, in the absence of Brilliant Black BN, the concentration-response relationship of the adenosine receptor agonist, NECA, was rightward shifted in the presence of 1 µM XAC-BY630 and 1 µM XAC. When repeated in the presence of Brilliant Black BN, the potency of 1 µM XAC-BY630 and 1 µM XAC was decreased in a concentration-dependent manner such that virtually no antagonism was observed in the presence of 500 µM Brilliant Black BN (Table 1). In summary, Brilliant Black BN caused a concentration-dependent decrease in the antagonism mediated by XAC-BY630 and XAC at both the adenosine-A_1 and -A_3 receptors.

	Brilliant Black concentration (µM)			
	0	**5**	**50**	**500**
Adenosine A_1 receptor				
NECA	-8.0 ± 0.3	-8.3 ± 0.3	-8.3 ± 0.3	-8.0 ± 0.1
+ 1 µM XAC-BY630	-7.3 ± 0.4	-7.7 ± 0.2	-7.9 ± 0.2	-8.0 ± 0.1
+ 1 µM XAC	-7.0 ± 0.2	-7.4 ± 0.3	-7.8 ± 0.4	-7.9 ± 0.1
Adenosine A_3 receptor				
NECA	-7.8 ± 0.2	-7.7 ± 0.2	-8.0 ± 0.2	-7.7 ± 0.1
+ 1 µM XAC-BY630	-6.7 ± 0.2	-6.5 ± 0.3	-7.2 ± 0.2	-7.5 ± 0.1
+ 1 µM XAC	-6.7 ± 0.3	-6.4 ± 0.3	-7.4 ± 0.1	-7.4 ± 0.2

Table 1: The potency ($LogEC_{50}$) NECA in the presence or absence of adenosine receptor antagonists and/or Brilliant Black. Values are mean ± S.E.M. of 3-10 experiments conducted in triplicate.

Briddon, S.J. *et al*. (2004) *P.N.A.S.*, **101**: 4673-4678
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