

THE EFFECT OF BRILLIANT BLACK BN ON AGONIST ANTAGONIST INTERACTIONS AT THE HUMAN ADENOSINE-A₁ AND -A₃ 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-A₁ and -A₃ receptors. Intracellular calcium mobilisation was measured using a fluorescence plate reader (FLEXstation, Molecular Devices). CHO cells (expressing either the human A₁ or A₃ 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-A₁ and -A₃ 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-A₁ and -A₃ receptors.

	Brilliant Black concentration (µM)			
	0	5	50	500
Adenosine A₁ 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 A₃ 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 (LogEC₅₀) 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

Roth A.L. *et al.* (2006) *J. Biol Chem.* **281**: 20809-20816