

CHARACTERIZATION AND PHOTOAFFINITY LABELING OF A_1 - ADENOSINE RECEPTORS IN COATED VESICLES FROM BOVINE BRAIN.

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ABSTRACT. The antagonist (3H) DPCPX exhibited a K_d of 0.4 nM at coated vesicles from bovine brain. Agonist competition for (3H) DPCPX binding revealed two affinity states for agonists. The photoaffinity probe ^{125}I -AHPIA specifically labelled a band with a molecular weight of 35 Kd.

Coated vesicles (CV) are involved in receptor mediated endocytosis of different receptors along with their ligands (1). Recently, it has been shown that a adenylate -cyclase coupled A_1 adenosine receptor exists in CV from bovine brain (2). We now present evidence that the A_1 receptors in CV are identical to those from brain membranes.

A_1 receptors in CV have been characterized by radioligand binding with (3H)DPCPX, an A_1 selective antagonist. A K_d of 0.4 nM was estimated, compared to 0.2 nM in bovine brain membranes. The B_{max} was 59 fmol/mg protein. Competition of agonists for (3H)DPCPX binding resulted in biphasic curves indicating the presence of two affinity states (Fig.1).

The biphasic competition curve of CCPA for (3H)DPCPX was shifted to the right by addition of 0.1 mM GTP. The curve was monophasic in the presence of GTP with a K_d value close to the low affinity K_d in the absence of GTP. This shows that A_1 receptors in CV are coupled to a G protein.

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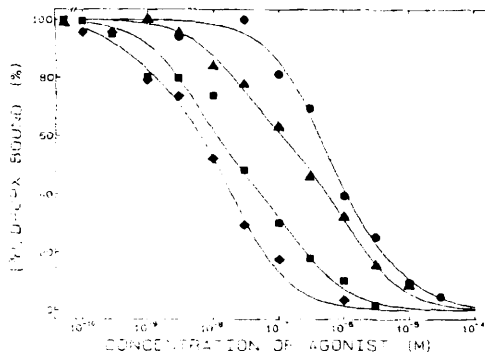


FIGURE 1. Competition of agonists for (^3H) - DPCPX binding. Shown is specific binding as % of control in the absence of agonist. Nonlinear curve fitting gave the following K_d values (nM) for the high and low affinity states, respectively: R-PIA (\blacklozenge) 0.3 and 7.6; CCPA (\blacksquare) 1.6 and 53; S-PIA (\blacktriangle) 4.7 and 205; NECA (\bullet) 143 and 2414.

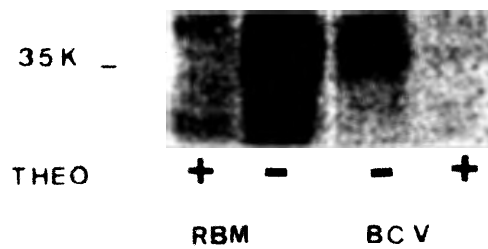


FIGURE 2. Photoaffinity labelling of A_1 adenosine receptors in bovine brain CV (BCV). Labelling of the band with 35 KD was inhibited in the presence of 1mM theophylline (THEO), indicating specific labelling. The same band was labelled in rat brain membranes (RBM).

Agonist radioligand binding further confirmed that A_1 receptors in CV are coupled to a G protein. The A_1 selective agonist (^3H) CCPA (3), showed high affinity binding with a K_d value of 2 nM and a B_{max} value of 36 fmol/mg protein. The agonist photoaffinity label ^{125}I -AHPIA (4) was specifically incorporated in a band with a molecular weight of 35 KD (Fig 2).

High affinity agonist binding confirmed that G protein-coupled A_1 -adenosine receptors exist in CV from bovine brain. ^{125}I -AHPIA was photoincorporated in a band of similar molecular weight as in brain membranes from different species. Thus, A_1 receptors in CV have similar characteristics compared to receptors in brain membranes.

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