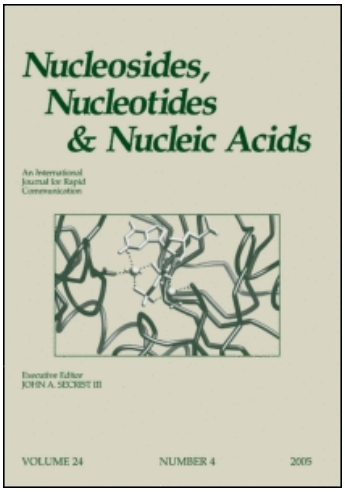

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Solubilized Rat Brain Adenosine Receptors Have Two Binding Sites For 1,3-Dipropyl-8-Cyclopentylxanthine

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SOLUBILIZED RAT BRAIN ADENOSINE RECEPTORS HAVE TWO BINDING SITES FOR 1,3-DIPROPYL-8-CYCLOPENTYLXANTHINE

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Abstract. The use of [^{3}H]-L-phenylisopropyladenosine ([^{3}H]-L-PIA) and 1,3-dipropyl-8-cyclopentylxanthine (DPCPX) in binding assays to solubilized preparations from rat brain membranes suggested the presence of an A_1, and a non-A_1 non-A_2 adenosine receptor may be equivalent to the A_3 subtype.

We studied the binding properties of [^{3}H]-L-PIA to solubilized preparations from rat brain membranes, and the inhibition of this binding by DPCPX.

Membranes were prepared and solubilized according to the method described by Helmke and Cooper[1]. The incubation was performed at 23° C, during 2 h, in presence of [^{3}H]-L-PIA (0.5-12.0 nM), adenosine deaminase (ADA) (1 U/ ml), BSA (1 mg/ ml) and $MgCl_2$ (2 mM), in a final volume of 100 ul. Non-specific binding was determined with 10 uM of nonlabelled L-PIA. Similar preparations were incubated at 23°C, during 3h, with [^{3}H]-L-PIA (2.5 nM) and DPCPX (0.1-300 nM).

The specific binding of [^{3}H]-L-PIA to the solubilized preparations was concentration-dependent. The Scatchard plot of this binding was biphasic suggesting the existence of two components. The computer LIGAND treatment data produced K_D values for the first and the second components of 0.24 nM and 3.56 nM, and number of binding sites (B_{Max}) of 0.28±0.03 pmol/ mg protein and 0.66±0.05 pmol/mg protein, respectively (n=5). The inhibition of the [^{3}H]-L-PIA binding by DPCPX gave sigmoidal competition curves (Fig. 1). The Hofstee plot generated from these data

showed a biphasic regression curve suggesting the presence of two high affinity binding sites for DPCPX. The K_i values for DPCPX obtained using non-linear curve fitting were 0.29±0.08 nM and 13.5±0.98 nM, and proportion of the two sites was about 20-30% and 70-80%, respectively.

The present results suggested the existence of two distinct adenosine receptors being the B_{Max} value for the first approximately half of the second. The K_i value for DPCPX found in the present work for the first affinity component (K_i=0.29 nM) is within the same range of the values obtained by others for the A_1-adenosine receptor[1,2]. The affinity value of DPCPX found for the second component of the binding is different either from the affinity values for A_1 ($K_i \leq$ 1.0 nM) or A_2 (K_i=340 nM) adenosine receptors. This value is in the same order of magnitude of the K_i values obtained by Alexander et al.[3] in mammalian cerebral cortical slices (i.e., 8-24 nM) and also in relatively good agreement with the value found by Sebastião and Ribeiro[4] for the receptor mediating inhibition of the transmitter release at the frog neuromuscular junction (i.e., 35 nM).

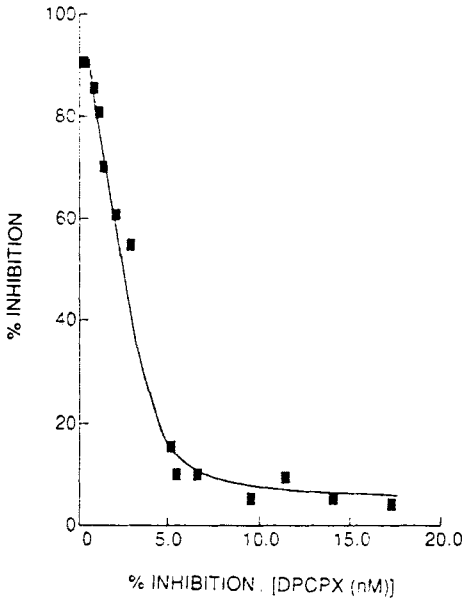

Fig.1 Hofstee plot generated from the inhibition of the [^{3}H]-L-PIA binding by DPCPX.

In summary the higher affinity binding site at the present work might correspond to the A_1-adenosine receptor and the lower is compatible with the presence of a non-A_1 non-A_2 receptor, may be equivalent to the A_3-adenosine receptor subtype.

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