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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 [^3H]-L-phenylisopropyladenosine ([^3H]-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 [^3H]-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 Cooper¹. The incubation was performed at 23°C, during 2 h, in presence of [^3H]-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 μl . Non-specific binding was determined with 10 μM of nonlabelled L-PIA. Similar preparations were incubated at 23°C, during 3h, with [^3H]-L-PIA (2.5 nM) and DPCPX (0.1-300 nM).

The specific binding of [^3H]-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 [^3H]-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

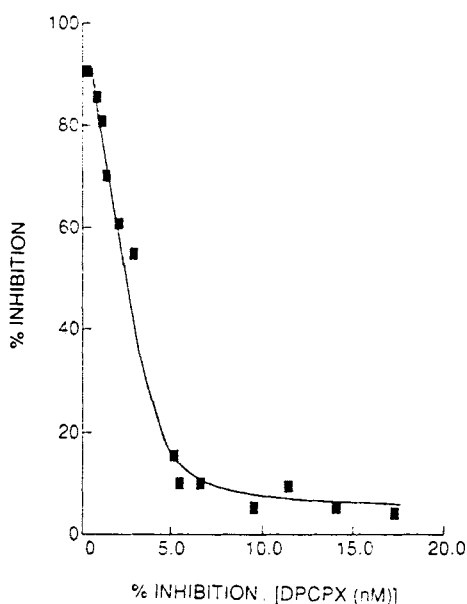


Fig.1 Hofstee plot generated from the inhibition of the [3 H]-L-PIA binding by DPCPX.

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 Ribeiro⁴ for the receptor mediating inhibition of the transmitter release at the frog neuromuscular junction (i.e., 35 nM).

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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