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2′,3′-Dideoxy-N^6-cyclohexyladenosine: an adenosine derivative with antagonist properties at adenosine receptors

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The 2′,3′-dideoxy analogue of the potent A_1 receptor agonist, N^6-cyclohexyladenosine (CHA), was synthesized as a potential antagonist for the A_1 adenosine receptor. In studies on adenylate cyclase 2′,3′-dideoxy-N^6-cyclohexyladenosine (ddCHA) did not show agonist properties at A_1 or at A_2 receptors. However, it antagonized the inhibition by R-PIA of adenylate cyclase activity of fat cell membranes via A_1 receptors with a K_i value of 13 μM. ddCHA competed for the binding of the selective A_1 receptor antagonist, [^{3}H]8-cyclopentyl-1,3-dipropylxanthine ([^{3}H]DPCPX), to rat brain membranes with a K_i value of 4.8 μM; GTP did not affect the competition curve. In contrast to the marked stereoselectivity of the A_1 receptor for the α- and the natural β-anomer of adenosine, the α-anomer of ddCHA showed a comparable affinity for the A_1 receptor (K_i value 13.9 μM). These data indicate that the 2′- and 3′-hydroxy groups of adenosine and its derivatives are required for agonist activity at and high affinity binding to A_1 adenosine receptors and for the distinction between the α- and β-forms.

Adenosine receptors; Adenylate cyclase; Adenosine receptor antagonists

1. Introduction

Adenosine regulates a large variety of physiological functions including the nervous and cardiovascular systems (reviewed in Gerlach and Becker, 1987). In general, it appears to represent a negative feed-back mediator, which serves to restore a balance between metabolic supply and demand (Newby, 1984). Most of its effects are mediated through specific membrane-bound receptors (reviewed in Lohse et al., 1988b). These receptors have been subdivided on biochemical and pharmacological grounds into A_1 and A_2 subtypes, of which the A_2 receptor mediates stimulation of adenylate cyclase, whereas the A_1 receptor mediates inhibition of adenylate cyclase and in some cell types opening of K^+ channels. The two subtypes are generally defined by their different affinities for agonists, with R-N^6-phenylisopropyladenosine (R-PIA) being more potent than 5′-N-ethylcarboxamidoadenosine (NECA) at A_1 receptors but being less potent at A_2 receptors (Londos et al., 1980), whereas methylxanthines such as theophylline are antagonists with equal affinity for both subtypes.

The structure-activity relationships at the A_1 receptor have been investigated by many authors using radioligand binding experiments and tests of receptor-mediated functions (see for example Hamilton et al., 1985; Schwabe et al., 1985; Daly et al., 1986; Cristalli et al., 1988). These studies

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have led to the development of agonists and antagonists with greater than 1000-fold selectivity for the A_1 receptor (Cristalli et al., 1988; Bruns et al., 1987; Lohse et al., 1987). However, much less is known about the requirements for agonist activity at the A_1 receptor. Studies with adenine (Ebert and Schwabe, 1973), 9-substituted adenine derivatives (Ukena et al., 1987) and ribose-modified adenosine analogues (Trost and Stock, 1977; Taylor et al., 1986) have pointed to the importance of the ribose moiety both for binding affinity and agonist effects. In the present study we report that removal of the 2′- and 3′-hydroxy groups of the potent full A_1 receptor agonist, N^6-cyclohexyladenosine (CHA), is sufficient to completely abolish its agonist activity.

2. Materials and methods

The synthesis of 2′,3′-β-dideoxy-N^6-cyclohexyladenosine (ddCHA) and its α-anomer is described elsewhere (Diekmann, 1986). 8-Cyclopentyl-1,3-[^{3}H]dipropylxanthine ([^{3}H]DPCPX) was prepared as described previously (Lohse et al., 1987, commercially available from Amersham). [α-^{32}P]ATP was obtained from Amersham Buchler, Braunschweig, FRG, and purified as described earlier (Lohse et al., 1988a). α-Adenosine was purchased from Sigma, Deisenhofen, FRG. Deoxycoformycine was a kind gift from Prof. Osswald, Gödecke, Freiburg, FRG.

Membranes from rat brain, rat fat cells and human platelets were prepared as outlined earlier (Lohse et al., 1987; Klotz et al., 1985). The adenylate cyclase activity of rat fat cell membranes and human platelet membranes was determined as described by Klotz et al. (1985). Binding of [^{3}H]DPCPX to rat brain membranes was measured as reported recently (Lohse et al., 1987). For binding assays involving adenosine as a competitor, membranes were pretreated with 0.2 U/ml adenosine deaminase as usual (Lohse et al., 1984) and the enzyme activity was blocked by the addition of 100 nM deoxycoformycine. The A_2 receptors of human platelet membranes were solubilized and separated from non-receptor [^{3}H]NECA binding sites by gel filtration. [^{3}H]NECA binding to the A_2 receptors was measured using pooled fractions containing the A_2 receptors (Lohse et al., 1988a).

Concentration-response curves were fitted to a modified Hill equation as described earlier (Lohse et al., 1986), and radioligand binding data were analyzed with the programme SCTFIT (De Lean et al., 1982). All curve-fitting procedures were carried out by using non-linear regression.

3. Results

The effects of ddCHA on adenosine receptors were investigated in adenylate cyclase experiments in which rat fat cell membranes were used as a model for the A_1 receptor and human platelet membranes were used as a model for the A_2 receptor. ddCHA did not display any agonist activity in either model in concentrations up to 1 mM. However, 100 μM ddCHA shifted the R-PIA inhibition curve for rat fat cell membrane adenylate cyclase to the right in a parallel manner, indicating competitive antagonism (fig. 1). The Schild equation gave a K_i value for ddCHA of 13 μM. The stimulation of human platelet membrane adenylate cyclase by NECA via A_2 receptors was only weakly affected by 100 μM ddCHA, giving a K_i value of about 280 μM. These data show that ddCHA acts as a pure antagonist at both A_1 and

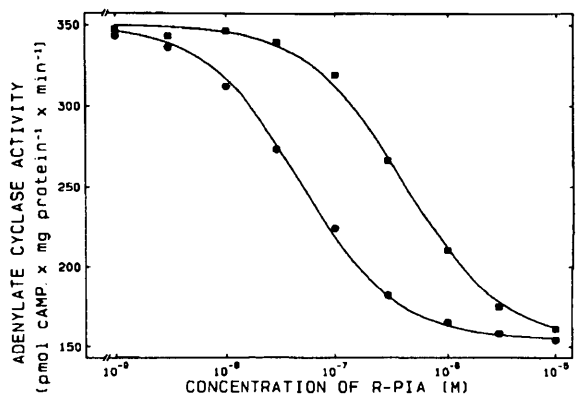

Fig. 1. Inhibition of rat fat cell adenylate cyclase by R-PIA in the absence (●) and presence of 100 μM ddCHA (■).

A_2 receptors, with a greater than 20-fold selectivity for the A_1 receptor.

Similar affinities of ddCHA for A_1 and A_2 adenosine receptors were observed in binding studies (table 1). ddCHA competed for the binding of [^{3}H]DPCPX to A_1 receptors of rat brain membranes with a K_i value of 4.8 μM, and for the binding of [^{3}H]NECA to solubilized A_2 receptors of human platelet membranes with a K_i value of 160 μM. The competition curve for [^{3}H]DPCPX binding had a slope factor of about 1 and was not affected by 100 μM GTP. Both observations confirm the antagonist properties of ddCHA (Lohse et al., 1984).

The α-anomer of ddCHA was somewhat less potent than ddCHA at A_1 receptors but was slightly more potent at A_2 receptors. This finding is in marked contrast to the pronounced stereoselectivity of adenosine receptors for adenosine and its α-anomer (fig. 2). Since binding assays with adenosine require the inactivation of adenosine deaminase, such experiments can only yield apparent K_i values due to the presence of endogenous adenosine. Based on these values, the rat brain A_1 receptor showed greater than 1000-fold preference for adenosine compared to α-adenosine, whereas ddCHA was only 3 to 4 times more potent than α-ddCHA. Similarly, the binding of [^{3}H]NECA to A_2 receptors was inhibited by adenosine with a K_i value of 190 nM and by α-adenosine with a K_i value of 80 μM. In contrast, α-ddCHA was even more potent than ddCHA in inhibiting [^{3}H]NECA binding to A_2 receptors (table 1).

TABLE 1

Affinities of ddCHA and α-ddCHA for A_1 and A_2 adenosine receptors.

Compound	K_i (μM)		A_1 selectivity $K_i(A_2)/K_i(A_1)$
	A_1 receptor	A_2 receptor	
Adenylate cyclase			
ddCHA	13	280	22
α-ddCHA	20	55	2.8
Radioligand binding			
ddCHA	4.8	160	33
α-ddCHA	14	45	3.2

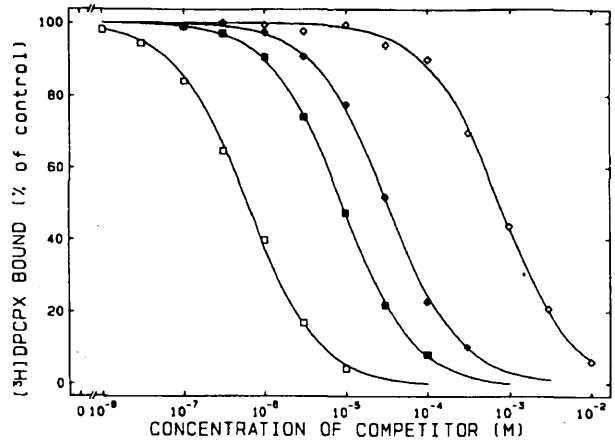

Fig. 2. Inhibition of [^{3}H]DPCPX binding to rat brain membranes by adenosine (□), α-adenosine (◇), ddCHA (■) and α-ddCHA (◆). After pretreatment, adenosine deaminase activity was blocked by addition of 100 nM deoxycoformycine. The apparent K_i values were: adenosine 220 nM, α-adenosine 350 μM, ddCHA 4.3 μM and α-ddCHA 17 μM.

4. Discussion

CHA is a potent full agonist at A_1 receptors (Cristalli et al., 1988). Removal of the 2′- and 3′-hydroxy groups results not only in a decrease of affinity, which is more marked for the A_1 than for the A_2 receptor, but also in a complete loss of agonist activity. Thus, ddCHA is a pure antagonist at both adenosine receptor subtypes, with a 20- to 30-fold selectivity for the A_1 receptor. Removal of the 3′-hydroxy group from R-PIA has been shown to result only in a moderate loss of affinity and the retention of at least partial agonist affinity (Taylor et al., 1986). Removal of the 2′-hydroxy group from adenosine (Trost and Stock, 1977) or R-PIA (Taylor et al., 1986) results in a marked loss of affinity, but some agonist affinity appears to remain (Trost and Stock, 1977). At the A_2 receptor of human fibroblasts, 3′-deoxyadenosine has been reported to act as a partial agonist and non-competitive inhibitor of the adenosine-induced increase of cAMP and 2′-deoxyadenosine has been reported to act as a non-competitive inhibitor (Bruns, 1980).

In addition to the loss of agonist activity, the removal of the two hydroxy residues completely abolishes the stereoselective recognition of the β- versus the α-glycosidic bond by both adenosine receptor subtypes. It seems likely, therefore, that the rest of the ribose moiety does not markedly contribute to receptor binding. This hypothesis is supported by the relatively high affinity reported for N^6-cyclohexyl-9-methyladenine (Ukena et al., 1987).

In summary, our results show that the 2′- and 3′-hydroxy groups are essential for agonist activity and for the recognition of the β- versus the α-anomer at adenosine receptors. ddCHA is an adenosine derivative that is an A_1-selective antagonist, and may be a useful tool in the investigation of the ligand binding site of the A_1 adenosine receptor.

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