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

Martin J. Lohse 1,*, Karl-Norbert Klotz 1, Elke Diekmann 2, Klaus Friedrich 2 and Ulrich Schwabe 1

1 Pharmakologisches Institut, Im Neuenheimer Feld 366, D-6900 Heidelberg, and 2 Institut für Organische Chemie und Biochemie, Alberstraße 21, D-7800 Freiburg, F.R.G.

Received 4 May 1988, revised MS received 4 August 1988, accepted 16 August 1988

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]HJ8-cyclopentyl-1,3-dipropylxanthine ([^3]HJDPCPX), 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.

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
have led to the development of agonists and antagonists with greater than 1000-fold selectivity for the \( \text{A}_1 \) 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 \( \text{A}_1 \) 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 \( \text{A}_1 \) receptor agonist, N\(^6\)-cyclohexyladenosine (CHA), is sufficient to completely abolish its agonist activity.

2. Materials and methods

The synthesis of 2',3',\( \beta \)-dideoxy-N\(^6\)-cyclohexyladenosine (ddCHA) and its \( \alpha \)-anomer is described elsewhere (Diekmann, 1986). 8-Cyclopentyl-1,3-[\( ^3 \text{H} \)]dipropylxanthine ([\( ^3 \text{H} \)]DPCPX) was prepared as described previously (Lohse et al., 1987, commercially available from Amersham). [\( \alpha-\text{\( ^32 \)P} \)]ATP was obtained from Amersham Buchler, Braunschweig, FRG, and purified as described earlier (Lohse et al., 1988a). \( \alpha \)-Adenosine was purchased from Sigma, Deisenhofen, FRG. Deoxycoformycin 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 adenylylate cyclase activity of rat fat cell membranes and human platelet membranes was determined as described by Klotz et al. (1985). Binding of [\( ^3 \text{H} \)]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 deoxycoformycin. The \( \text{A}_2 \) receptors of human platelet membranes were solubilized and separated from non-receptor [\( ^3 \text{H} \)]NECA binding sites by gel filtration. [\( ^3 \text{H} \)]NECA binding to the \( \text{A}_2 \) receptors was measured using pooled fractions containing the \( \text{A}_2 \) 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 adenylylate cyclase experiments in which rat fat cell membranes were used as a model for the \( \text{A}_1 \) receptor and human platelet membranes were used as a model for the \( \text{A}_2 \) receptor. ddCHA did not display any agonist activity in either model in concentrations up to 1 mM. However, 100 \( \mu \)M ddCHA shifted the R-PIA inhibition curve for rat fat cell membrane adenylylate 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 \( \mu \)M. The stimulation of human platelet membrane adenylylate cyclase by NECA via \( \text{A}_2 \) receptors was only weakly affected by 100 \( \mu \)M ddCHA, giving a \( K_i \) value of about 280 \( \mu \)M. These data show that ddCHA acts as a pure antagonist at both \( \text{A}_1 \) and

![Fig. 1. Inhibition of rat fat cell adenylylate cyclase by R-PIA in the absence (*) and presence of 100 \( \mu \)M ddCHA (\( \bullet \)).](attachment:fig1.png)
A₂ receptors, with a greater than 20-fold selectivity for the A₁ receptor.

Similar affinities of ddCHA for A₁ and A₂ adenosine receptors were observed in binding studies (table 1). ddCHA competed for the binding of [³H]DPCPX to A₁ receptors of rat brain membranes with a Kᵢ value of 4.8 µM, and for the binding of [³H]NECA to solubilized A₂ receptors of human platelet membranes with a Kᵢ value of 160 µM. The competition curve for [³H]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 A₁ receptors but was slightly more potent at A₂ 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 Kᵢ values due to the presence of endogenous adenosine. Based on these values, the rat brain A₁ 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 [³H]NECA to A₂ receptors was inhibited by adenosine with a Kᵢ value of 190 nM and by α-adenosine with a Kᵢ value of 80 µM. In contrast, α-ddCHA was even more potent than ddCHA in inhibiting [³H]NECA binding to A₂ receptors (table 1).

4. Discussion

CHA is a potent full agonist at A₁ 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 A₁ than for the A₂ 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 A₁ 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 A₂ 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 N6-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 A1-selective antagonist, and may be a useful tool in the investigation of the ligand binding site of the A1 adenosine receptor.

Acknowledgement

This study was supported by a grant from the Deutsche Forschungsgemeinschaft.

References


Diekmann, E., 1986, Potenzielle Adenosinantagonisten: Die Synthese von 2',3'-Dideoxynucleosiden, PhD-thesis (Faculty of Chemistry and Pharmacy, Freiburg/Breisgau).


Newby, A.C., 1984, Adenosine and the concept of 'retaliatory metabolites', Trends Biochem. Sci. 9, 42.


