

Short communication

2-Chloro-N⁶-cyclopentyladenosine: a highly selective agonist at A₁ adenosine receptors

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Summary. 2-Chloro-N⁶-cyclopentyladenosine (CCPA) was synthesized as a potential high affinity ligand for A₁ adenosine receptors. Binding of [³H]PIA to A₁ receptors of rat brain membranes was inhibited by CCPA with a K_i-value of 0.4 nM, compared to a K_i-value of 0.8 nM for the parent compound N⁶-cyclopentyladenosine (CPA). Binding of [³H]NECA to A₂ receptors of rat striatal membranes was inhibited with a K_i-value of 3900 nM, demonstrating an almost 10,000-fold A₁-selectivity of CCPA.

CCPA inhibited the activity of rat fat cell membrane adenylate cyclase, a model for the A₁ receptor, with an IC₅₀-value of 33 nM, and it stimulated the adenylate cyclase activity of human platelet membranes with an EC₅₀-value of 3500 nM. The more than 100-fold A₁-selectivity compares favourably with a 38-fold selectivity of CPA. Thus, CCPA is an agonist at A₁ adenosine receptors with a 4-fold higher selectivity and 2-fold higher affinity than CPA, and a considerably higher selectivity than the standard A₁ receptor agonist R-N⁶-phenylisopropyladenosine (R-PIA). CCPA represents the agonist with the highest selectivity for A₁ receptors reported so far.

Key words: Adenosine receptors – Adenylate cyclase

Introduction

Adenosine appears to be a negative feed-back mediator which influences a number of physiological functions (reviewed by Gerlach and Becker 1987). Its primary function seems to be the restoration of a balance between metabolic supply and demand (Newby 1984). Most of its effects are mediated via specific membrane-bound receptors (for a review see Lohse et al. 1988a). These receptors have been subdivided into an A₁ and an A₂ subtype (Van Calcar et al. 1978; Londos et al. 1980), of which the A₂ receptor mediates a stimulation of adenylate cyclase, whereas the A₁ receptor mediates an inhibition of adenylate cyclase as well as in some cell-types an opening of K⁺-channels (see for example Kurachi et al. 1986). The two subtypes are generally defined by their different affinities for agonists, with R-N⁶-phenylisopropyladenosine (R-PIA) being more potent than 5'-N-ethylcarboxamidoadenosine (NECA) at A₁, but less

potent at A₂ receptors, whereas methylxanthines such as theophylline are antagonists with equal affinities for both subtypes.

Several agonists with selectivity for the A₁ receptor have been described in the past. However, their selectivity has been found to be insufficient in order to demonstrate an inhibition of adenylate cyclase activity in tissues that contain also stimulatory A₂ receptors (Freissmuth et al. 1987). Consequently, agonists with higher A₁-selectivity are required to establish the role of adenylate cyclase in A₁ receptor-mediated effects. In a recent investigation of 1-deaza adenosine analogues we found that a 2-chloro substitution of 1-deaza-adenosines leads to an enhancement of A₁-selectivity in the case of a N⁶-cyclopentyl-substituent, whereas it reduces the selectivity of other N⁶-substituted 1-deaza-adenosines (Cristalli et al. 1988). 2-Chloro-N⁶-cyclopentyl-1-deazaadenosine proved to be 3-fold more selective than N⁶-cyclopentyladenosine (CPA), the most selective A₁ receptor agonist reported thus far (Moos et al. 1985). However, the 1-deaza modification resulted in a loss of affinity compared to CPA. In order to avoid this reduction of affinity, we undertook the synthesis and characterization of the analogue without the 1-deaza modification, 2-chloro-N⁶-cyclopentyladenosine (CCPA).

Materials and methods

The synthesis of 2-chloro-N⁶-cyclopentyladenosine (CCPA) was performed following a procedure previously described (Cristalli et al. 1986) starting from 2,6-dichloro-9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)purine and cyclopentylamine. A ¹H-NMR spectrum was obtained with a Varian EM-390 90 MHz spectrometer. All exchangeable protons were confirmed by addition of D₂O. The microanalytical results are indicated by atomic symbols and are within ± 0.4% of theoretical values: ¹H-NMR (DMSO-d₆) δ 1.37–2.33 (large m, 9H, H cyclopentyl), 3.64 (m, 2H, CH₂-5'), 3.98 (m, 1H, H-4'), 4.17 (m, 1H, H-3'), 4.54 (m, 1H, H-2'), 5.88 (d, J = 6 Hz, 1H, H-1'), 8.28 (d, J = 7.5 Hz, 1H, NH), 8.41 (s, 1H, H-8). Anal. (C₁₅H₂₀ClN₅O₄) C, H, N.

Membranes from whole rat brain, rat striatum, rat fat cells and human platelets were prepared as outlined earlier (Lohse et al. 1987). The adenylate cyclase activity of rat fat cell and human platelet membranes was determined as described by Klotz et al. (1985). Binding of [³H]PIA (1 nM) to rat brain membranes was measured as described (Lohse et al. 1984), and binding of [³H]NECA (10 nM) to rat striatal membranes was performed according to Bruns et al. (1986)

Abbreviations. CCPA, 2-chloro-N⁶-cyclopentyladenosine; CPA, N⁶-cyclopentyladenosine; NECA, 5'-N-ethylcarboxamidoadenosine; PIA, N⁶-phenylisopropyladenosine

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Table 1. Affinities of R-PIA, CPA and CCPA for A₁ and A₂ adenosine receptors

Radioligand binding	A ₁ receptor K _i (nM)	A ₂ receptor K _i (nM)	A ₁ -selectivity K _i (A ₂)/K _i (A ₁)
R-PIA	1.3 (1.1–1.6)	730 (690–770)	560
CPA	0.8* (0.6–1.0)	2000* (1400–2900)	2500
CCPA	0.4* (0.2–0.7)	3900* (2500–4700)	9750
Adenylate cyclase	A ₁ receptor IC ₅₀ (nM)	A ₂ receptor (EC ₅₀ (nM)	A ₁ -selectivity EC ₅₀ /IC ₅₀
R-PIA	40 (22–72)	950 (650–1400)	24
CPA	58 (24–140)	2200* (1500–3200)	38
CCPA	33 (22–51)	3500* (3200–3800)	107

EC₅₀- and IC₅₀-values (concentrations causing 50% of the respective maximal effects) were calculated from the inhibition of rat fat cell adenylate cyclase (A₁ receptor) and the stimulation of human platelet adenylate cyclase (A₂ receptor). K_i-values were measured in competition experiments using [³H]PIA binding to rat brain membranes (A₁ receptor) and [³H]NECA binding to rat striatal membranes (A₂ receptor). Values are geometric means and 95% confidence limits of 5 independent experiments. Asterisks denote significant differences ($p < 0.001$, Student's *t*-test) versus corresponding values obtained with R-PIA

with the modifications described earlier (Lohse et al. 1987). Data were analyzed by non-linear curve-fitting procedures as described (Lohse et al. 1987). All binding data were adequately fitted with a one site model.

Results

The effects of CCPA on adenosine receptors were first investigated in radioligand binding experiments and compared with those of the parent compound CPA and the standard A₁ receptor agonist R-PIA (Table 1). CCPA inhibited the binding of the A₁ receptor-selective agonist [³H]PIA to rat brain membranes with a K_i-value of 0.4 nM, and was thus two times more potent than CPA, and three times more potent than R-PIA. In contrast, binding of [³H]NECA to A₂ receptors of rat striatal membranes was inhibited by CCPA with a K_i-value of 3900 nM, which was two-fold higher than the K_i-value of CPA. In both cases, significant differences between the K_i-values of CCPA and R-PIA were obtained. These values show an almost 10,000-fold selectivity of CCPA for A₁ vs. A₂ receptors, which compares favourably with the selectivities of CPA and R-PIA.

The functional selectivity of CCPA was determined in studies of the adenosine receptor-mediated modulation of adenylate cyclase activity, using the inhibition in rat fat cell membranes in the presence of 10 μM forskolin as a model for the A₁ receptor, and the stimulation in human platelet membranes as a model for the A₂ receptor. CCPA inhibited the adenylate cyclase activity of rat fat cell membranes with an IC₅₀-value of 33 nM. The maximal inhibition was the

same (48 ± 3%) as by CPA (52 ± 4%) and R-PIA (53 ± 10%). CCPA, CPA and R-PIA stimulated the adenylate cyclase activity of human platelet membranes with EC₅₀-values of 3.5 μM, 2.2 μM and 950 nM, respectively. The maximal stimulation by all three compounds was less than that obtained with NECA: whereas NECA stimulated the activity by up to 250 ± 30%, the maximal stimulation obtained with CCPA was 150 ± 30%, with CPA 130 ± 25%, and with R-PIA 135 ± 15%. Thus, CCPA appears to be a full agonist at A₁ receptors, but a partial agonist at A₂ receptors, with a 100-fold A₁-selectivity in adenylate cyclase experiments.

Discussion

The investigation of several 1-deaza-analogues of adenosine (Cristalli et al. 1988) led us to the conclusion that N⁶-cyclopentyl- and 2-Cl-substitutions of 1-deaza-adenosine are additive in producing selectivity for A₁ adenosine receptors. The present results show that the same is true with adenosine itself, thus avoiding the loss of affinity caused by the 1-deaza-modification: CCPA has an almost 10,000-fold selectivity in binding assays, and a more than 100-fold selectivity in adenylate cyclase studies. In both sets of experiments, its selectivity is 3- to 4-fold higher than that of the parent compound CPA, and considerably higher than that of R-PIA. In addition, its affinity for the A₁ receptor is even higher than that of CPA. Thus, the selectivity of CCPA is similar to that of 2-chloro-N⁶-cyclopentyl-1-deazaadenosine (Cristalli et al. 1988), but its affinity is four times higher.

The difference between the selectivity of agonists as determined in adenylate cyclase experiments compared to that obtained in radioligand binding studies has been observed in earlier studies. It is most likely explained by the formation of a high affinity state of the A₁ receptor for agonists in the absence of GTP (i.e. under the usual conditions of the radioligand binding assay). This causes a discrepancy between the K_i-values of radioligand binding to the A₁ receptor and the IC₅₀-values of adenylate cyclase inhibition via the A₁ receptor by a factor of about 100, which corresponds to the difference of the K_i-values of the high (absence of GTP) and the low (presence of GTP) affinity states (Lohse et al. 1984). No such differences between data from adenylate cyclase and radioligand binding experiments were observed in the case of the affinities for the A₂ receptor, which agrees with the minor effects of low concentrations of GTP on the binding of [³H]NECA to A₂ receptors (Lohse et al. 1988b).

Whereas the inhibition of adenylate cyclase in rat fat cell membranes suggests that CCPA is a full agonist at the A₁ receptor, it has only partial agonist properties at the A₂ receptor of human platelet membranes. The same observation has been made for other N⁶-substituted adenosine derivatives (Hüttemann et al. 1984; Cristalli et al. 1988).

In summary, CCPA appears to be the most selective agonist at A₁ receptors reported so far. It should allow the sensitive detection of A₁ receptor-mediated effects.

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References

- Bruns RF, Lu GH, Pugsley TA (1986) Characterization of the A₂ adenosine receptor labeled by [³H]NECA in rat striatal membranes. *Mol Pharmacol* 29:331–346
- Cristalli G, Grifantini M, Vittori S, Klotz K-N, Lohse MJ (1986) Synthesis of 2-azido-(R)-N⁶-p-hydroxyphenylisopropyladenosine (R-AHPA) as a potential photoaffinity probe for A₁ adenosine receptors. *Nucleos Nucleot* 5:213–222
- Cristalli G, Franchetti P, Grifantini M, Vittori S, Klotz K-N, Lohse MJ (1988) Adenosine receptor agonists: Synthesis and biological evaluation of 1-deaza analogues of adenosine derivatives. *J Med Chem* (in press)
- Freissmuth M, Hausleithner V, Tuisl E, Nanoff C, Schütz W (1987) Glomeruli and microvessels of the rabbit kidney contain both A₁- and A₂-adenosine receptors. *Naunyn-Schmiedeberg's Arch Pharmacol* 335:438–444
- Gerlach E, Becker BF (1987) Topics and perspectives in adenosine research. Springer, Berlin Heidelberg New York Tokyo
- Hüttemann E, Ukena D, Lenschow V, Schwabe U (1984) R_a-Adenosine receptors in human platelets. Characterization by 5'-N-ethylcarboxamido[³H]adenosine binding in relation to adenylate cyclase activity. *Naunyn-Schmiedeberg's Arch Pharmacol* 325:226–233
- Klotz K-N, Cristalli G, Grifantini M, Vittori S, Lohse MJ (1985) Photoaffinity labeling of A₁ adenosine receptors. *J Biol Chem* 260:14659–14664
- Kurachi Y, Nakajima T, Sugimoto T (1986) On the mechanism of activation of muscarinic K⁺ channels by adenosine in isolated atrial cells: involvement of GTP-binding proteins. *Pflügers Arch* 407:264–274
- Lohse MJ, Lenschow V, Schwabe U (1984) Two affinity states of R₁ adenosine receptors in brain membranes: Analysis of guanine nucleotide and temperature effects on radioligand binding. *Mol Pharmacol* 26:1–9
- Lohse MJ, Klotz K-N, Lindenborn-Fotinos J, Reddington M, Schwabe U, Olsson RA (1987) 8-Cyclopentyl-1,3-dipropylxanthine (DPCPX) – a selective high affinity antagonist radioligand for A₁ adenosine receptors. *Naunyn-Schmiedeberg's Arch Pharmacol* 336:204–210
- Lohse MJ, Klotz K-N, Schwabe U, Cristalli G, Vittori S, Grifantini M (1988a) Pharmacology and biochemistry of adenosine receptors. In: Melchiorre C, Gianella M (eds) Recent advances in receptor chemistry. Elsevier, Amsterdam, pp 107–121
- Lohse MJ, Elger B, Lindenborn-Fotinos J, Klotz K-N, Schwabe U (1988b) Separation of solubilized A₂ adenosine receptors of human platelets from non-receptor [³H]NECA binding sites by gel filtration. *Naunyn-Schmiedeberg's Arch Pharmacol* 337:64–68
- Londos C, Cooper DMF, Wolff J (1980) Subclasses of external adenosine receptors. *Proc Natl Acad Sci USA* 77:2551–2554
- Moos WH, Szotek DS, Bruns RF (1985) N⁶-Cycloalkyladenosines. Potent A₁-selective adenosine agonists. *J Med Chem* 28:1383–1384
- Newby AC (1984) Adenosine and the concept of "retaliatory metabolites". *Trends Biochem Sci* 9:42–44
- Van Calcar D, Müller M, Hamprecht B (1978) Adenosine inhibits the accumulation of cyclic AMP in cultured brain cells. *Nature (Lond)* 276:839–841

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