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Short communication

2-Chloro-N^6-cyclopentyladenosine, adenosine A_1 receptor agonist, antagonizes the adenosine A_3 receptor

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Abstract

The potent adenosine A_1 receptor agonists, N^6-cyclopentyladenosine (CPA) and 2-chloro-N^6-cyclopentyladenosine (CCPA), were studied in Chinese hamster ovary (CHO) cells expressing the human adenosine A_3 receptor. CPA, but not CCPA, induced phosphoinositide turnover. CPA inhibited forskolin-stimulated cyclic AMP production (EC_{50} value of 242 ± 47 nM). CCPA competitively antagonized the effects of agonist Cl-IB-MECA (2-chloro-N^6-(3-iodobenzyl)-5′ -*N*-methylcarbamoyladenosine) with K_B value of 5.0 nM. CPA competition curves versus the A_3 antagonist radioligand [^{3}H]PSB-11 (8-ethyl-4-methyl-2-phenyl-(8*R*)-4,5,7,8-tetrahydro-1*H*-imidazo[2.1-*i*]purin-5-one) were right-shifted four-fold by 100 μM GTP, which had no effect on binding of CCPA or the antagonist MRS 1220 (*N*-[9-chloro-2-(2-furanyl)[1,2,4]triazolo[1,5-*c*]quinazolin-5-yl]benzene-acetamide). Thus, CCPA is a moderately potent antagonist (K_i = 38 nM) of the human A_3 adenosine receptor. Published by Elsevier Science B.V.

Keywords: Adenosine derivative; Purine; cAMP; Phospholipase C; Guanine nucleotide

1. Introduction

N^6-cyclopentyladenosine (CPA) and 2-chloro-N^6-cyclopentyladenosine (CCPA) were originally synthesized as high affinity ligands for adenosine A_1 receptors (Moos et al., 1985; Lohse et al., 1988) and have been used widely to study the receptor binding and function in vitro and in isolated organs (Monopoli et al., 1994; Shryock et al., 1998). 2-Chloro and other 2-position substituents on adenosine analogues were previously noted to increase affinity of agonists at various adenosine receptor subtypes without affecting efficacy (Hutchison et al., 1990; Kim et al., 1994). CPA and CCPA are among the most potent agonists for adenosine A_1 receptors reported so far (Lohse et al., 1988; Klotz et al., 1998), with binding K_i values at the high affinity site of the human homologue of 2.25 and 0.83 nM, respectively (Klotz et al., 1998). These adenosine derivatives have weak or moderate binding affinities at human adenosine A_{2A} or A_3 receptors, respectively (Salvatore et al., 1993). CCPA was shown to activate adenosine A_{2A} receptors (Shryock et al., 1998). However, it previously was unknown whether CCPA acted as an agonist or an antagonist of the adenosine A_3 receptor, as no indication that CCPA activates the adenosine A_3 receptor has been reported. It is often assumed that adenosine derivatives that activate one receptor subtype would likely activate other subtypes at concentrations at which binding is observed. In the course of studying the activation of human A_3 adenosine receptors, we found that CCPA bound to the human A_3 adenosine receptor with high affinity but did not activate the receptor. The present study demonstrated the distinct effects of CPA and CCPA on the activation of the human adenosine A_3 receptor with the surprising finding that CCPA is a moderately potent antagonist of the human adenosine A_3 receptor.

2. Materials and methods

2.1. Materials

CPA, CCPA, MRS 1220 (*N*-[9-chloro-2-(2-furanyl)[1,2,4]triazolo[1,5-*c*]quinazolin-5-yl]benzene-acetamide), and GTP were purchased from Sigma (St. Louis, MO). Myo-[^{3}H]inositol (20 Ci/mmol) was obtained from

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American Radiolabeled Chemicals (St. Louis, MO). [^{3}H]PSB-11 (8-ethyl-4-methyl-2-phenyl-(8*R*)-4,5,7,8-tetrahydro-1*H*-imidazo[2.1-*i*]purin-5-one, 53 Ci/mmol) and [^{3}H]cyclic AMP (40 Ci/mmol) were from Amersham Pharmacia Biotech (Buckinghamshire, UK). Cl-IB-MECA (2-chloro-N^6-(3-iodobenzyl)-5′ -*N*-methylcarbamoyladenosine) was obtained from the National Institute of Mental Health's Chemical Synthesis and Drug Supply Program.

2.2. Pharmacological methods

Chinese hamster ovary (CHO) cells expressing recombinant human A_3 adenosine receptors were cultured and membrane suspension prepared as described in Gao et al. (2001).

Radioligand binding were carried out by methods of Gao et al. (2001). Briefly, membranes (100 μg protein) were incubated with 5 nM [^{3}H]PSB-11 at 25 °C in a total assay volume of 400 μl for 60 min. Nonspecific binding was measured in the presence of 10 μM Cl-IB-MECA. For the experiments in the presence of GTP, the procedures were similar to our previously described (Gao et al., 2000). Cyclic AMP levels were measured with a competitive protein binding method (Nordstedt and Fredholm, 1990; Gao et al., 2001). Determination of inositol phosphates was as previously described (Chen et al., 2001). Binding and functional parameters were estimated using GraphPAD Prism software (GraphPAD, San Diego, CA, USA).

3. Results

We studied functional effects of CPA and CCPA at human adenosine A_3 receptor expressed in CHO cells. It has been demonstrated that the adenosine A_3 receptor is coupled to adenylyl cyclase as well as phospholipase C (Abbracchio et al., 1995; Chen et al., 2001). We found CPA to be a full agonist in stimulation of phospholipase C in intact CHO cells expressing the human adenosine A_3 receptor. CPA induced phosphoinositide turnover in a concentration-dependent manner with an EC_{50} of 223 ± 66 nM ($n = 3$), while CCPA up to a concentration of 10 μM elicited no stimulation (data not shown). In another functional assay, CPA inhibited forskolin-stimulated cyclic AMP production in CHO cells in a dose-dependent manner, as in a previous study (Klotz et al., 1998), corresponding to an EC_{50} value of 242 ± 47 nM ($n = 3$). CCPA, however, up to a concentration of 10 μM had no effect on cyclic AMP production under the same conditions.

Since CCPA was shown using an agonist radioligand to bind to the human adenosine A_3 receptor with a K_i value of 42 nM (Klotz et al., 1998), a further possibility remained that this adenosine derivative was an antagonist. We studied the effects on adenosine A_3 receptor agonist-induced inhibition of cyclic AMP production in A_3 receptor-expressing CHO cells. Antagonism by CCPA of the effect of the potent adenosine A_3 receptor agonist Cl-IB-MECA was demonstrated. Fixed concentrations of CCPA shifted the Cl-IB-MECA dose–response curve to the right in a concentration-dependent manner (Fig. 1A). A Schild analysis (Arunlakshana and Schild, 1959) was carried out on the antagonism (Fig. 1B) corresponding to a K_B value of 5.0 ± 1.2 nM ($n = 3$). The magnitude of the Cl-IB-MECA affinity shift induced by CCPA was linear with CCPA concentration, suggesting competitive antagonism.

The differential effects of CPA and CCPA on the human adenosine A_3 receptor were further demonstrated utilizing radioligand binding in a functional assay based on the GTP-shift method (Lorenzen et al., 1996; Jacobson et al., 1997; Van Tilburg et al., 1999). [^{3}H]PSB-11 is a novel adenosine A_3 receptor-selective radioligand introduced by Müller (2001). CCPA displaced this radioligand with a K_i value of 38 nM (Table 1). GTP (100 μM) caused a fourfold shift to the right of the curve representing CPA competition for [^{3}H]PSB-11 binding, but it had no effect on the competition

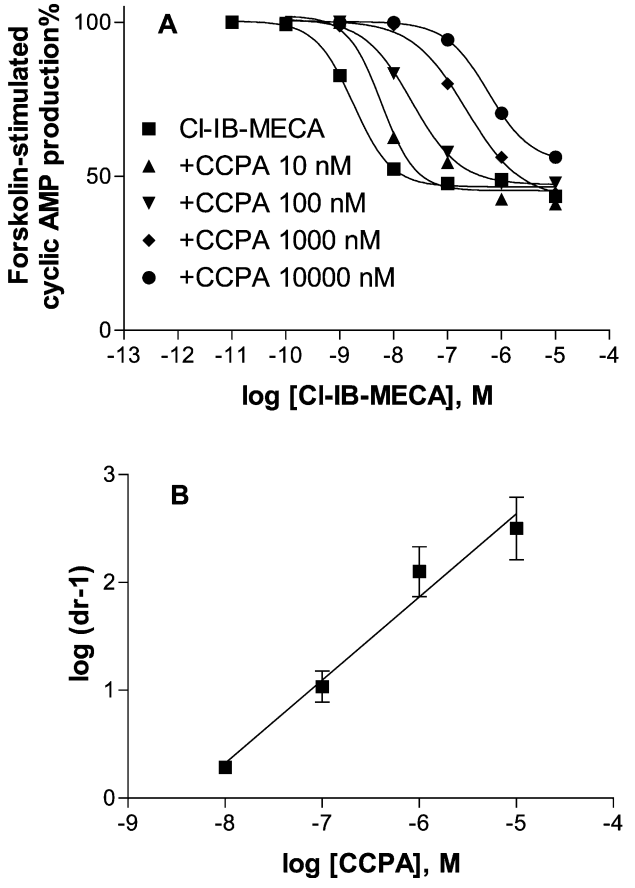

Fig. 1. Effects of the adenosine derivative CCPA on the inhibition of cyclic AMP production induced by the agonist Cl-IB-MECA in human A_3 adenosine receptor-expressing CHO cells (A) and Schild analysis (B) of the data. The procedures used were described as in Pharmacological methods. The data shown in A were derived from one experiment performed in duplicate and are typical of three independent experiments giving similar results. The data shown in B were expressed as mean ± standard error from three independent experiments. The K_B value listed in the text was calculated from three independent experiments.

Table 1
Affinity of three ligands at human A_3 receptors in the absence and presence of a guanine nucleotide[a]

	K_i (nM)		Shift (fold)
	Control	+GTP (100 μM)	
CPA	72 ± 12	310 ± 43[b]	4.3
CCPA	38 ± 6	34 ± 7	0.9
MRS 1220	1.2 ± 0.1	1.2 ± 0.2	1.0

[a] Binding was carried out in membranes (80 μg protein) of CHO cells stably expressing recombinant human A_3 adenosine receptors. The concentration of [^{3}H]PSB-11 was 5 nM. Data were expressed as mean ± S.E.M. from three independent experiments.
[b] $P < 0.05$ compared with control.

curves of CCPA and the selective adenosine A_3 receptor antagonist MRS 1220 (Jacobson et al., 1997). This confirmed the adenosine A_3 receptor agonism by CPA and indicated that both CCPA and MRS 1220 acted as antagonists in this assay.

4. Discussion

CCPA is a commonly used adenosine A_1 receptor agonist; however, its functional properties at the adenosine A_3 receptor subtype have not been previously characterized. Some studies have utilized both CCPA and Cl-IB-MECA to activate selectively adenosine A_1 and A_3 receptors, respectively. For example, these derivatives have been used to study the cardiac protect effects of adenosine receptor activation in cardiac myocyte cultures (Strickler et al., 1996). CCPA has been used in pharmacological studies at high concentrations that might antagonize the adenosine A_3 receptor (Cargnoni et al., 1999), thus the present findings may limit applications of this compound in certain pharmacological models. Although the discovery that an agonist at one receptor subtype antagonizes at another subtype of the same receptor class is commonplace for adrenergic receptors (Jasper and Insel, 1992), it is unusual for adenosine derivatives.

The structural difference between these two adenosine analogues is simply the presence of a 2-chloro group. Previously, such a group was shown to enhance binding affinity for various adenosine receptor subtypes (Kim et al., 1994); however, effects on efficacy by this group alone were not previously reported. Recently 2-substitution was reported to reduce against efficacy (Van Tilburg et al., 2001). Evidently, the reduction of efficacy induced by the 2-chloro group in this paired set of analogues was total. The effect of a 2-chloro group was not universal, since the pair of IB-MECA and Cl-IB-MECA, both full agonists at the adenosine A_3 receptor, completely deviated from this observation. Thus, the effect on efficacy of a 2-chloro group must depend on other substituents on the molecule, such as at the N^6 position (here cyclopentyl). In a previous study (Jacobson et al., 1995), we unsuccessfully sought to convert the agonist IB-MECA into an adenosine A_3 receptor antagonist by truncating the ribose moiety. In fact, the present study accomplishes that goal with the agonist CPA using a more easily attainable structural modification.

It will be necessary to examine the behavior of other structural pairs of adenosine agonists to assess the generality of the present findings. In future studies, it will also be instructive to vary the species under examination and the level of overexpression of the A_3 receptor, which may affect the ability to detect residual agonism by CCPA.

In a separate study, we have described the effects of single amino acid mutations of the human adenosine A_3 receptor on the affinity and potency of diverse adenosine agonists (Gao et al., in press). It will be interesting to explore the structural basis for the lack of activation of the adenosine A_3 receptor by CCPA, which appears to be a major qualitative distinction between adenosine A_1/A_{2A} receptors and the adenosine A_3 receptor. The existence of pairs of closely related agonists and antagonists may aid in the elucidation of the conformational factors responsible for receptor activation (Sharma et al., 2001; Nandanan et al., 2000).

In conclusion, this is the first identification of a moderately potent antagonist of the adenosine A_3 receptor based on an agonist nucleoside structure. By this strategy, it may be possible to further modify the structure of CCPA in order to diminish adenosine A_1 receptor affinity and therefore provide adenosine A_3 receptor selectivity.

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