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8-(3-Chlorostyryl)caffeine (CSC) is a selective A_2-adenosine antagonist in vitro and in vivo

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An adenosine antagonist, 8-(3-chlorostyryl)caffeine (CSC), was shown previously to be 520-fold selective for A_{2a}-adenosine receptors in radioligand binding assays in the rat brain. In reversing agonist effects on adenylate cyclase, CSC was 22-fold selective for A_{2a} receptors in rat pheochromocytoma cells (K_b 60 nM) vs. A_1 receptors in rat adipocytes (K_b 1.3 μM). Administered i.p. in NIH mice at a dose of 1 mg/kg, CSC shifted the curve for locomotor depression elicited by the A_{2a}-selective agonist APEC to the right (ED_{50} value for APEC shifted from 20 μg/kg i.p. to 190 μg/kg). CSC had no effect on locomotor depression elicited by an ED_{50} dose of the A_1-selective agonist CHA. CSC alone at a dose of 5 mg/kg stimulated locomotor activity by 22% over control values. Coadministration of CSC and the A_1-selective antagonist CPX, both at non-stimulatory doses, increased activity by 37% ($P < 0.001$) over CSC alone, suggesting a behavioral synergism of A_1- and A_2-antagonist effects in the CNS.

Adenosine receptor; Xanthine; Locomotor activity; Dopamine; Adenylate cyclase

1. INTRODUCTION

Adenosine agonists act as locomotor depressants through A_1 and A_{2a} receptor subtypes [1–3]. An in vivo synergistic relationship between activation of A_1 and A_{2a} receptors in the brain was demonstrated using the selective agonists CHA (A_1; N^6-cyclohexyladenosine) and APEC (A_{2a}; 2-[(2-aminoethylamino)carbonylethyl phenylethylamino]-5′-*N*-ethylcarboxamidoadenosine)[3]. The second messenger generally associated with adenosine receptors is cyclic AMP, the formation of which is inhibited by A_1 receptor activation and stimulated by A_{2a} receptor activation [12]. Ion channels and phospholipid turnover also can be modulated through A_1 receptors [4]. The stimulant effects of caffeine and other methylxanthines are related, at least in part, to their ability to block adenosine receptors [4], although the interpretation is complicated by action of these agents as phosphodiesterase inhibitors [4]. Furthermore, certain potent and A_1-selective adenosine antagonists, such as CPX (8-cyclopentyl-1,3-dipropylxanthine), do not stimulate locomotor activity [5].

Highly A_2-selective antagonists, various 1,3,7-trialkyl-8-styrylxanthine derivatives, have only recently been synthesized [6]. We have explored the structure–activity relationships of 70 members of this class of compounds [7] and found that the highest selectivity in binding assays was obtained with 8-(3-chlorostyryl)caffeine (CSC; 1,3,7-trimethyl-8-(3-chlorostyryl)xanthine). CSC was 520-fold selective comparing affinity at A_{2a} receptors in the rat striatum (K_i 54 nM) and A_1 receptors in the rat cerebral cortex (K_i 28 μM). In the present study it is shown that CSC also is highly selective in functional adenylate cyclase assays and in vivo with respect to locomotor activity.

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2. EXPERIMENTAL

2.1. *Agents*

CSC and APEC were synthesized as described [7,8]. All other xanthines and adenosine analogs are commercially available.

2.2. *Biochemical activity*

Antagonism of NECA-elicited stimulation of adenylate cyclase via an A_{2a} receptor in rat pheochromocytoma (PC12) cell membranes or in human platelets was assayed as described [10]. Antagonism of N^6-phenylisopropyladenosine-elicited inhibition of adenylate cyclase via an A_1 receptor in rat adipocyte membranes was assayed as described [10]. K_b values were calculated using the Schild equation from the ratio of EC_{50} values for agonist in the presence and absence of antagonist.

2.3. *Locomotor activity*

Adult male mice of the NIH (Swiss) strain weighing 25–30 g were housed in groups of 10 animals per cage with a light–dark cycle of 12:12 h. The animals were given free access to standard pellet food and water and were acclimatized to laboratory conditions for 24 h prior to testing. Each animal was used only once in the activity monitor.

Locomotor activity of individual animals was studied in an open field using a Digiscan activity monitor (Omnitech Electronics Inc., Columbus, OH) equipped with an IBM-compatible computer. The computer-tabulated measurements represent multivariate locomotor analysis with specific measures, such as simultaneous measurements

of ambulatory, rearing, stereotypical, and rotational behaviors Data was collected in the morning, for three consecutive intervals of 10 min each, and analyzed separately and as a group. Statistical analysis was performed using the Student's *t*-test. The results are reported as mean ± standard error for each point. All drugs were dissolved in a vehicle consisting of a 20:80 v/v mixture of Alkamuls EL-620 (Rhône-Poulenc, Cranbury, NJ) and phosphate-buffered saline, except for CSC which was dissolved initially in DMSO and diluted in at least 20 vols. of vehicle. Drugs were administered i.p. in a volume corresponding to 5 ml/kg body weight. Where applicable, the antagonist was injected 10 min before the agonist. ED_{50} values were determined using regression analysis on the InPlot software (GraphPAD, San Diego, CA).

3. RESULTS

In reversing adenosine agonist effects on adenylate cyclase (Table I), CSC was 22-fold selective for A_{2a} receptors in rat pheochromocytoma (PC12) cells vs. A_1 receptors in rat adipocytes. CSC displayed a lower potency in adenylate cyclase effects at A_{2a} receptors in human platelets (K_b 260 nM) than at rat A_{2a} receptors in PC12 cells (K_b 60 nM). This probably reflects the species difference: large differences in potency of xanthines at A_{2a}-adenosine receptors of different species have been noted previously [11].

The locomotor effects in mice of CSC alone or in combination with the potent and A_{2a}-selective agonist APEC [3] were examined. CSC administered i.p. at a maximum soluble dose of 1 mg/kg was found to nearly completely reverse the locomotor depression elicited by APEC at its previously determined [3] ED_{50} of 16 μg/kg i.p. (Fig. 1A). A dose of CSC of 5 mg/kg (injected as a suspension, since the solubility was exceeded at 1 mg/ml of injection vehicle) was found to cause significant locomotor stimulation by 22% over vehicle control value. The total distance traveled in CSC animals was 4,223 ± 496 cm/30 min (n = 13) vs. 3,449 ± 198 cm/30 min (n = 8) in controls. This stimulation was most pronounced (56% increase vs. control) in the last 10 min of the 30 min monitoring period. Since CSC was not very efficacious in stimulating locomotor activity at the highest tested dose, the ED_{50} for CSC alone was not determined. The concurrent administration of a 16 μg/kg dose of APEC with 5 mg/kg CSC had no effect on the locomotor activity. The drug combination resulted in a total distance traveled of 3,949 ± 284 cm/30 min (n = 14). This level of locomotor activity represents a 73% increase vs. APEC alone with 2,277 ± 229 cm/30 min (n = 13).

CSC (5 mg/kg) had no effect on locomotor depression elicited by the potent A_1 agonist CHA at its determined ED_{50} value of 100 μg/kg i.p. Coadministration of both drugs resulted in a total distance traveled of 2,029 ± 250 cm/30 min (n = 8) vs. 2,090 ± 438 cm/30 min (n = 9) for the CHA control.

Dose–response curves for locomotor depression by APEC in the absence and presence of CSC are presented in Fig. 1B. The ED_{50} for locomotor depression elicited by APEC was right-shifted from 20 μg/kg i.p. to 190 μg/kg following administration of 1 mg/kg CSC.

The A_1-selective antagonist CPX was administered alone and in combination with CSC (Fig. 2). CPX alone resulted in a total distance traveled of 3,035 ± 330 cm/30 min (n = 14); i.e. a minimal depressant effect on locomotor activity compared to control. CSC alone (1 mg/kg) had no significant effect on locomotor activity, with a total distance traveled of 3,550 ± 230 cm/30 min (n = 19). However, the combination of the two antagonists, each at a subthreshold dose, stimulated locomotor activity by 37% ($P < 0.001$) over CSC alone (total distance traveled of 4,861 ± 243 cm/30 min, n = 9), suggesting a synergism of A_1- and A_2-antagonist effects in the CNS. Following coadministration, the average dis-

Table I

Receptors affinities and effects of various xanthines on adenosine agonist-elicited inhibition (A_1) or stimulation (A_2) of adenylate cyclase

Compd.	Inhibition of binding (K_i; μM)		Adenylate cyclase (K_b; μM)			Behavioral stimulation[c]
	Rat cortex[a] A_1	Rat striatum[b] A_{2a}	Rat adipocytes A_1	Human platelets A_{2a}	Rat PC12 cells A_{2a}	
Caffeine	44	41	59	30	37	+++ (30)
DMPX	45	16	94	4.0	9 6	+++ (10)
CPT	0.024	1 4	n.d.	0.14	n.d.	++[d] (10)
CPX	0 0009	0.47	0.0006	0.14	0 25	− (1)
CSC	28	0.054	1.32 ± 0 26	0.26 ± 0.07	0.060 ± 0.014	+ (5)

[a] vs. agonist ligand [^{3}H]N^6-phenylisopropyladenosine.

[b] vs. agonist ligand [^{3}H]*N*-ethylcarboxamidoadenosine, except vs. agonist ligand [^{3}H]CGS 21680 for CSC

[c] Degree of stimulation relative to vehicle control during a 30 min monitoring period, indicated by − (< 10%), + (10–25%), ++ (25–50%), and +++ (50–150%), with a typical dose (mg/kg, i.p.) shown in parentheses

[d] Stimulatory effect disappears within 20 min post-injection [5].

n.d. = not determined. Values are means from prior publications [7,9,10,12–14] or are means ± S.E.M. (n = 3–4).

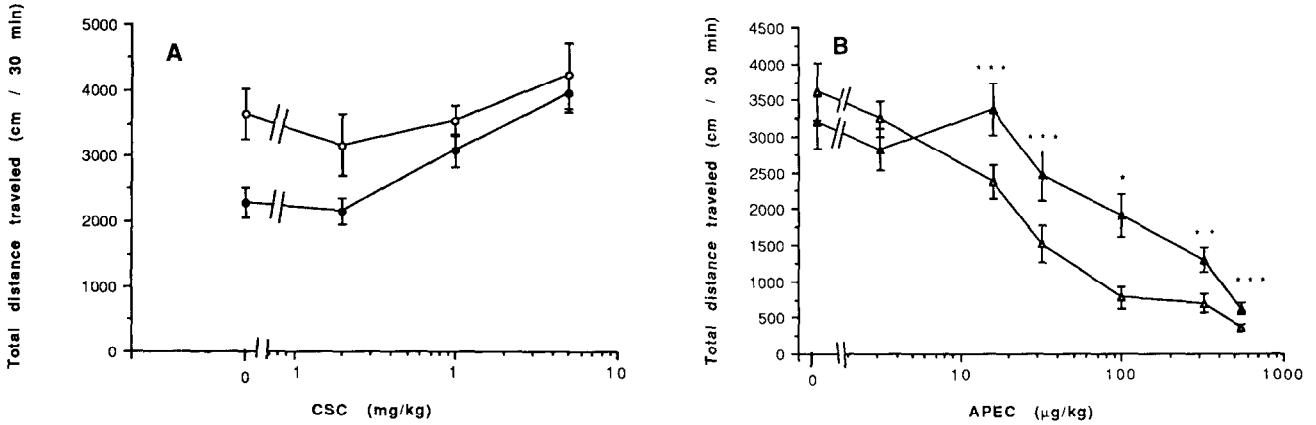

Fig. 1. (A) Locomotor activity in male NIH Swiss mice (6 week) by the A_2-selective adenosine antagonist CSC alone (○) or in the presence of the A_2-selective agonist APEC at 16 μg/kg (●). (B) Locomotor depression in mice by APEC alone (△) or in the presence of CSC at 1.0 mg/kg (▲). $n = 6$–19. $^*P < 0.005$; $^{**}P < 0.01$; $^{***}P < 0.025$

tance per move was increased by approximately 30%, and clockwise and anti-clockwise rotations were increased in the range of 30–60% (data not shown).

4. DISCUSSION

An A_2 antagonist suitable for in vivo use has been lacking. Previously, a low affinity antagonist 3,7-dimethyl-1-propargylxanthine (DMPX) was reported to be A_2 selective, by less than one order of magnitude [13]. It was relatively weak in blocking the in vivo effects of CHA compared to those of NECA, suggesting some A_2 selectivity. Several non-xanthine antagonists of the triazoloquinazoline class, including CGS 15943, are A_2-selective but also by only one order of magnitude [16]. The locomotor activity of several members of this class was described previously [17]. A triazoloquinoxaline derivative, CP66,713, was found to be 12-fold selective in binding assays at rat brain A_{2a} vs. A_1 receptors [18]. Low selectivity, interspecies differences in affinity, and low water solubility precluded extensive use of this compound. In one study, partial antagonism of A_2 depression of locomotor activity was achieved in vivo using CP66,713 [3]. At the same dose CP66,713 had no effect on A_1 depression of locomotor activity.

The 8-styrylxanthine derivatives reported by Shimada et al. [6] appear to be among the most A_2-selective antagonists currently known. A further structure activity study [7] introduced CSC as a particularly A_2-selective member of that class of xanthines. Since at the highest dose administered there was essentially no effect on the locomotor depression elicited by CHA, CSC is a functionally specific antagonist at A_{2a} vs. A_1 receptors in mice in vivo. In future studies with CSC it will be useful to determine the blood and brain concentrations of the drug and effect on cardiovascular function [6]. The selectivity of CSC for A_{2a} vs. A_{2b} (low affinity for agonists) receptors is yet to be established. It should be noted however that a physiological role has not yet been assigned to the low affinity A_{2b} receptor.

A relationship between the striatal dopaminergic and the adenosine A_2 systems has been proposed (reviewed in [19]). Activation of A_{2a} receptors inhibits a dopaminergic pathway in the striatum. D_2-dopamine receptors and A_{2a} receptors are colocalized on the subset of GABAergic neurons in the striatum innervating the globus pallidus and expressing enkephalin. Thus, an A_2 antagonist would be expected to enhance dopaminergic striatopallidal transmission. The other class of striatal GABAergic neurons, those expressing substance P, are located in the striatonigral pathway. An A_1 antagonist would not have a direct postsynaptic action on striatopallidal neurons, but might still affect both striatopallidal and striatonigral dopaminergic pathways by enhancing the release of dopamine in the striatum. Activation of presynaptic A_1 receptors is associated with the inhibition of release of stimulatory neurotransmitters in the CNS [19].

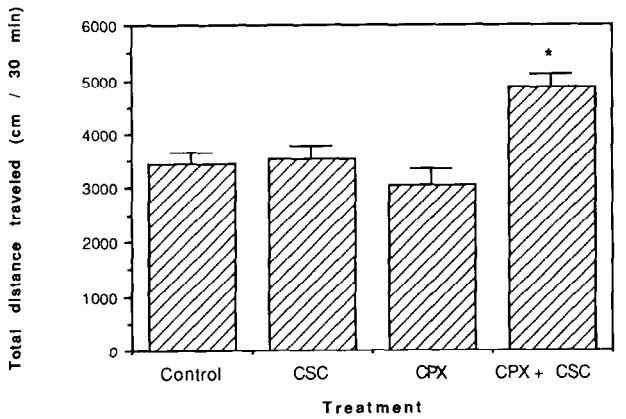

Fig. 2. Synergism of an A_1-selective antagonist (CPX, 0.25 mg/kg, i.p.) and an A_2-selective antagonist (CSC, 1.0 mg/kg, i.p.) in stimulating locomotor activity in mice. $n = 9$–19. $^*P < 0.001$ vs. CSC alone.

Selective A_1 and A_{2a} antagonists alone are either non-stimulatory or weakly stimulatory in locomotor activity (Table I), but the combination (as we have shown for subthreshold doses of CSC and CPX) causes substantial stimulation (Fig. 2). An increase in rotational movement, seen with the combination of A_1 and A_{2a} antagonists, is also observed with maximal stimulant doses of caffeine (unpublished results). This suggests the possibility that enhancement of dopaminergic action by blocking both presynaptic (A_1) and postsynaptic (A_{2a}) mechanisms might be required for substantial locomotor stimulation by xanthines. The pronounced enhancement of locomotor activity by non-selective xanthines (Table I) such as caffeine and theophylline [1,3] is consistent with this view. The moderate, but transient locomotor stimulation by CPT (8-cyclopentyltheophylline) may result from its non-selectivity in vivo at high doses (Table I). The synergistic behavioral depressant effects of A_1 agonists in combination with A_{2a} agonists [3] is also consonant with this proposal.

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