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Short communication

CGS 21680 exerts marked antidystonic effects in a genetic model of paroxysmal dyskinesia

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Abstract

The effect of the adenosine A_{2A} receptor agonist CGS 21680 (2-carboxyethyl)phenylethylamino]-5′-*N*-ethylcarbonyamido-adenosine) on severity of dystonia was examined in genetically dystonic hamsters which exhibit attacks of dystonic and choreoathetotic disturbances in response to mild stress. CGS 21680 significantly reduced the severity of dystonia (0.5, 1.0 and 2.0 mg/kg i.p.). The marked antidystonic effects of CGS 21680 in the hamster model suggest that this compound may represent an interesting candidate for the therapy of paroxysmal dystonia. Furthermore, the present data indicate that the precipitating effect of caffeine in patients with paroxysmal dystonia is probably due to its adenosine receptor antagonistic action.

Keywords: Adenosine receptor agonist; Basal ganglia disorder; Dystonia; Movement disorders

1. Introduction

Adenosine modulates motor activity by interactions with the dopaminergic system within the basal ganglia. Activation of adenosine A_{2A} receptors, which are highly concentrated in the striatum, inhibit the activity of dopamine D_2 receptors (Ferre et al., 1997; Fuxe et al., 1998). Adenosine receptor antagonists potentiate and adenosine agonists inhibit the hyperlocomotion caused by dopamine receptor agonists (Ferre et al., 1997). Therefore, adenosine receptor agonists and antagonists have been suggested to provide new therapeutic approaches for basal ganglia disorders (Popoli et al., 1994; Ferre et al., 1997).

Idiopathic dystonias, characterized by involuntary contractions of opposing muscles, frequently causing twisting movements or abnormal postures, are regarded as a basal ganglia disorder. The pathogenesis of various types of dystonias seems to be heterogeneous (Fahn et al., 1998). In a type of dystonia, the paroxysmal non-kinesiogenic dystonic choreoathetosis, attacks of generalized dystonic and choreoathetotic movements last up to several hours and can be provoked by stress and by consuming coffee or tea (Demirkiran and Jankovic, 1995), i.e., by factors which are known to increase the striatal dopaminergic activity (Abercrombie et al., 1989; Fredholm, 1995). As shown by several previous examinations, mutant dystonic hamsters (gene symbol dt^{sz}), which show all characteristics of this type of idiopathic hereditary dyskinesia (Demirkiran and Jankovic, 1995; Richter and Löscher, 1998), are useful for preclinical drug testing and for giving insights into the pathophysiology of this movement disorder. In line with the hypothesis that dystonia is due to dopaminergic dysfunctions, previous pharmacological and neurochemical studies indicated that dopaminergic overactivity is critically involved in the manifestation of dystonic attacks in dt^{sz} hamsters (Nobrega et al., 1996; Rehders et al., 2000).

With regard to the prodystonic effects of caffeine, known as non-selective adenosine $A_{1/2A}$ receptor antagonist (Fredholm, 1995), in patients with paroxysmal dystonia and mutant hamsters (Demirkiran and Jankovic, 1995; Richter and Löscher, 1998), the aim of the present study was to examine whether CGS 21680 (2-carboxyethyl)-phenylethylamino]-5′-*N*-ethyl-carbonyamido-adenosine) attenuates the stress-induced dystonic symptoms in dt^{sz} hamsters.

2. Materials and methods

The three groups of 7–9 dt^{sz} mutant hamsters used for the present study were obtained by selective breeding as

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described previously (Löscher et al., 1989; Richter and Löscher, 1998). In these mutant hamsters, dystonic attacks can be induced by handling or mild environmental stimuli. The dystonic attacks were induced by a triple stimulation technique (Löscher et al., 1989), i.e. stressful stimuli consisting of (1) taking the animal from its home cage and placing it on a balance, (2) intraperitoneal injection of vehicle (0.3% Tween 80) or of CGS 21680 (injection volume 5 ml/kg), and (3) placement of the animal in a new plastic cage. Thereafter, *dt*^{sz} hamsters develop a sequence of abnormal movements and postures, allowing to rate the severity of dystonia by the following score-system (Löscher et al., 1989; Richter and Löscher, 1998): stage 1, flat body posture; stage 2, disturbed gait with hyperextended forepaws; stage 3, hyperextended hindlimbs so that the animals appear to walk on tiptoes; stage 4, twisting movements and loss of balance; stage 5, hindlimbs hyperextended caudally; stage 6, immobilisation in a twisted, hunched posture with hind- and forelimbs tonically extended forward. Since the individual maximum stage of dystonia is usually reached within 3 h, the hamsters were observed for 3 h after triple stimulation. During this period the severity of dystonia, the latencies to the different stages and the side effects were noticed. The rater of the severity of dystonia was blind to the treatment condition of the animals. In a further group of five *dt*^{sz} hamsters, the body temperature (rectal) was measured every 30 min after administration of CGS 21680 (0.5 mg/kg). All drug experiments were done with hamsters between 35 and 41 days of age. Pre- and post-drug control trials were undertaken 2 days before and 2 days after drug testing. CGS 21680 (Biotrend, Cologne) was dissolved in 0.3% Tween 80. The significance of differences in severity of dystonia and in latencies to onset between control and

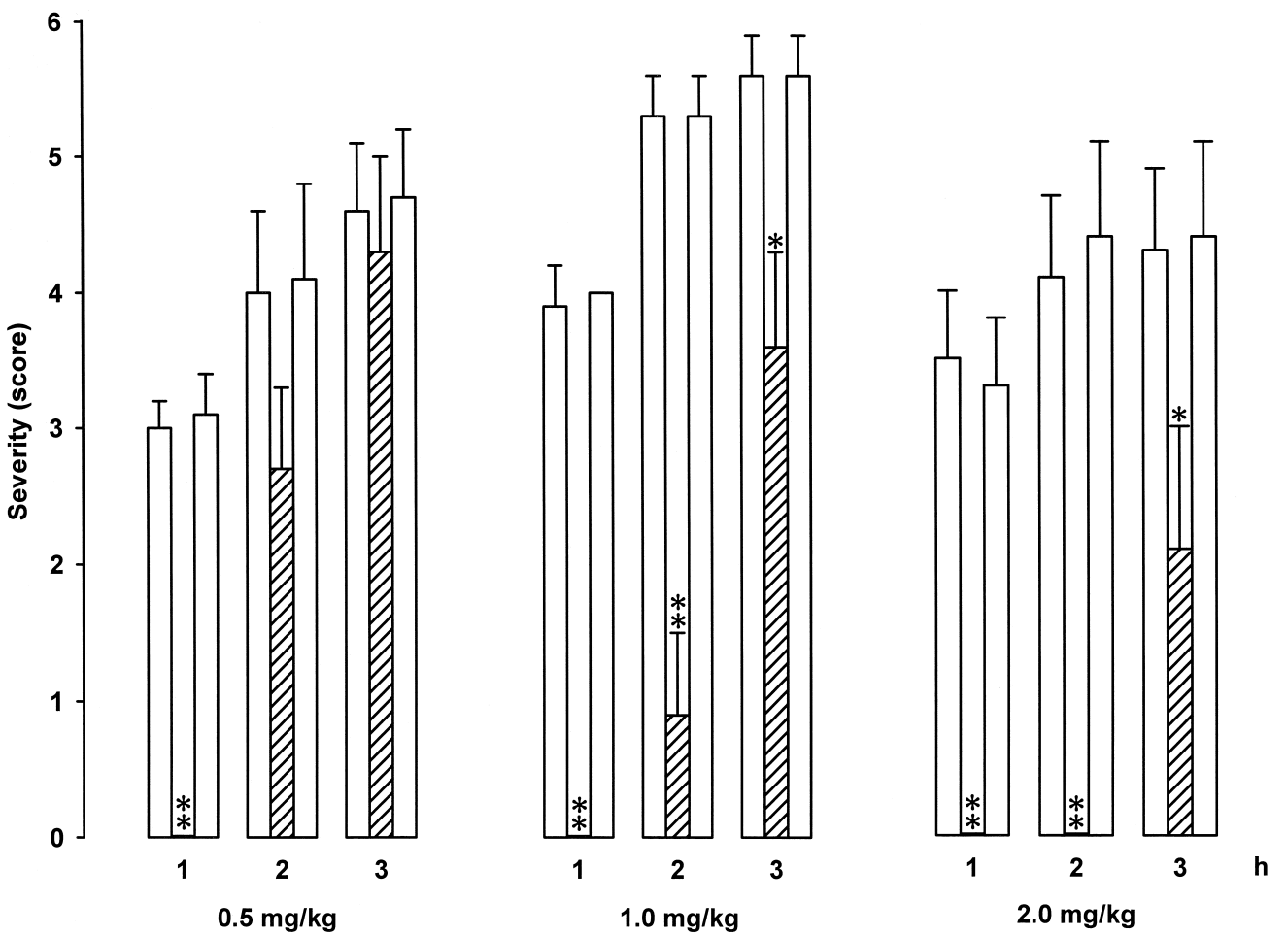

Fig. 1. Effect of CGS 21680 on severity of dystonia in mutant hamsters after intraperitoneal injections of 0.5, 1.0 and 2.0 mg/kg. Usually, the individual maximum severity of dystonia is reached within 3 h after induction of dystonia by triple stimulation including the i.p. injection of drugs (hatched bars) or vehicle for pre- and post-drug controls (open bars). The figure shows the average of the maximum individual severity scores of dystonia reached within the 1st, 2nd and 3rd hour after administration of CGS 21680 or vehicle, reflecting the progression of dystonia in *dt*^{sz} hamsters after treatment with the active compound and control recordings. Control recordings were undertaken 2 days before (pre-drug control) and 2 days after (post-drug control) the drug trial. Asterisks indicate significant improvement of dystonia in comparison to the pre- and post-drug control ($^{*}P < 0.05$; $^{**}P < 0.01$). Data are shown as means ± S.E. of seven (0.5 mg/kg), nine (1 mg/kg) or eight (2 mg/kg) dystonic hamsters. Absence of S.E. bars indicates that all hamsters had reached the same severity.

Table 1
Effects of CGS 21680 on latency to onset of dystonic attacks in genetically dystonic hamsters

Dose (mg/kg)	Latency on			*n*
	Pre-drug	Drug	Post-drug	
0.5	7.0±0.8	85.0±8.3[a]	8.0±0.8	7
1.0	8.0±1.1	134.3±10.3[b]	8.0±1.1	9
2.0	5.5±2.9	148.0±6.9[c]	8.5±2.1	8

Latency was determined as the time to the first unequivocal signs of dystonic attacks (stage 2). Data are shown as means±S.E. of the number of animals indicated (*n*).

[a]Significance to pre-drug and post-drug controls at $P < 0.05$.

[b]Significance to pre-drug and post-drug controls at $P < 0.01$.

[c]At a dose of 2.0 mg/kg only four *dt*^{sz} hamsters reached stage 2 during the 3-h observation period.

drug trials was calculated by the Friedman test and post hoc by the Wilcoxon signed rank test for paired replicates.

3. Results

As shown in Fig. 1, CGS 21680 exerted striking antidystonic effects. The significant increase of the latency to onset of dystonia (Table 1) reflects a fast onset of the antidystonic action of CGS 21680. At a dose of 2 mg/kg, CGS 21680 completely prevented the induction of dystonic attacks in mutant hamsters during the first 2 h of observation. Four animals of this treated group did not exhibit any dystonic symptoms during the whole 3-h period of observation (see Table 1). A significant decrease of the severity of dystonia (Fig. 1) and a significant increase of the latency to onset of dystonia (Table 1) was also observed after administration of 0.5 and 1.0 mg/kg.

Five minutes after treatment with 2.0 mg/kg, the hamsters exhibited a marked reduction of spontaneous locomotor activity, but determinations of the descent latency in a block test, i.e., the time in which the hamsters maintained the forelimbs on a block (6-cm high), did not disclose any catalepsy. The hypolocomotion lasted for about 140 min. At lower, but still antidystonic doses, the motor depressant effects were less marked (1.0 mg/kg) or unequivocal (0.5 mg/kg). As determined in a group of five mutant hamsters, moderate hypothermia (−1°C) was observed 30 and 60 min after administration of CGS 21680 (0.5 mg/kg).

4. Discussion

The present data demonstrate for the first time that the adenosine A_{2A} receptor agonist CGS 21680 exerts a pronounced improvement of paroxysmal dyskinesia, i.e., of paroxysmal dystonic choreoathetosis. Thus, the precipitating effects of caffeine in patients with this type of paroxysmal dystonia (Demirkiran and Jankovic, 1995) and in the genetic hamster model (Richter and Löscher, 1998) are likely due to the adenosine A_{2A} receptor antagonistic action of this methylxanthine. The stimulant effect of caffeine on locomotor activity has been shown to be mediated through its blockade of adenosine A_{2A} receptors (El Yacoubi et al., 2000). However, caffeine also antagonizes adenosine A_1 receptors (Fredholm, 1995). Both A_1 and A_{2A} receptor blockade can contribute to enhanced dopaminergic activity (Fuxe et al., 1998), which may be essential for the manifestation of paroxysmal dystonia (see below). Therefore, the present finding of a marked antidystonic effect of CGS 21680 should give rise to further experiments with A_1 receptor agonists and selective adenosine receptor antagonists in mutant hamsters.

As suggested by Ferre et al. (1997), the present results indicate that adenosine receptor agonists could be interesting candidates for the treatment of movement disorders which are related to striatal dopaminergic overactivity. Although the pathophysiology of different types of dystonia is still unknown, this heterogeneous group of movement disorders has been suggested to be due to striatal dopaminergic dysfunctions (Todd and Perlmutter, 1998). In mutant dystonic hamsters, several previous studies indicated that GABAergic disinhibition leads to an enhanced dopaminergic activity in the striatum (Richter and Löscher, 1998). Activation of dopamine D_2 receptors by systemic and also by intrastriatal injections of the dopamine D_2 receptor agonist quinpirole was found to cause a significant aggravation of dystonia in *dt*^{sz} hamsters (Rehders et al., 2000). Therefore, a striatal dopaminergic hyperactivity obviously plays a crucial role in the manifestation of dystonic attacks in *dt*^{sz} hamsters (Rehders et al., 2000). This is substantiated by the present finding of a significant antidystonic effect of CGS 21680, because adenosine A_{2A} receptor agonists are known to inhibit dopamine D_2 receptor activity (Ferre et al., 1997; Fuxe et al., 1998).

As observed in the present study in mutant hamsters, adenosine A_{2A} receptor agonists are known to cause depressant effects on locomotor activity and hypothermia similar to neuroleptics (Fuxe et al., 1998), which may limit the suitability for clinical use. Furthermore, hypotensive effects due to vasodilatation can be provoked by both CGS 21680 and neuroleptics (Ongini and Fredholm, 1996). Nevertheless, in types of dystonia in which neuroleptics exert beneficial effects, such as in paroxysmal dystonia (Fahn, 1995; Löscher and Fredow, 1992), adenosine A_{2A} receptor agonists may provide advantages. While long-term treatment with neuroleptics bears the risk to cause tardive dyskinesia (Marsden and Quinn, 1990), examinations of the A_{2A}/D_2 receptor interactions indicated that adenosine A_{2A} receptor agonists may be effective in the therapy of tardive dyskinesias (Fuxe et al., 1998). Furthermore, in *dt*^{sz} hamsters the antidystonic effects of CGS 21680 found in the present experiments were more marked than those of neuroleptics, such as haloperidol, shown in previous studies (Löscher and Fredow, 1992; Richter and Löscher, 1993). Thus, adenosine A_{2A} receptor agonists are possibly

interesting candidates for medical treatment of the often intractable dystonias, particularly for the therapy of paroxysmal dystonia.

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