



# Effects of adenosine receptor agonists and antagonists in a genetic animal model of primary paroxysmal dystonia

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**1** Recent studies have shown beneficial effects of an adenosine A<sub>2A</sub> receptor agonist in *dt<sup>sz</sup>* mutant hamsters, an animal model of paroxysmal dystonia, in which stress and consumption of coffee can precipitate dystonic attacks. This prompted us to examine the effects of adenosine receptor agonists and antagonists on severity of dystonia in *dt<sup>sz</sup>* hamsters in more detail.

**2** The non-selective adenosine A<sub>1</sub>/A<sub>2A</sub> receptor antagonists, caffeine (10–20 mg kg<sup>-1</sup> i.p.) and theophylline (10–30 mg kg<sup>-1</sup> s.c.), worsened the dystonia in *dt<sup>sz</sup>* hamsters.

**3** Aggravation of dystonia was also caused by the selective adenosine A<sub>1</sub>/A<sub>2A</sub> antagonist CGS 15943 (9-chloro-2-(2-furyl)[1,2,4]triazolo[1,5-c]quinazolin-5-amine) at a dose of 30 mg kg<sup>-1</sup> i.p. and by the adenosine A<sub>1</sub> antagonist DPCPX (8-cyclopentyl-1,3-dipropylxanthine; 20–30 mg kg<sup>-1</sup> i.p.), while the A<sub>2</sub> antagonist DMPX (3,7-dimethyl-1-propargylxanthine; 2–4 mg kg<sup>-1</sup> i.p.) and the highly selective A<sub>2A</sub> antagonist ZM 241385 (4-(2-[7-amino-2-(2-furyl)[1,2,4]triazolo[2,3-a][1,3,5]triazin-5-ylamino]ethyl)phenol; 2–5 mg kg<sup>-1</sup> i.p.) failed to exert any effects on dystonia.

**4** In contrast to the antagonists, both the adenosine A<sub>1</sub> receptor agonist CPA (N<sup>6</sup>-cyclopentyladenosine; 0.1–1.0 mg kg<sup>-1</sup> i.p.) and the A<sub>2A</sub> agonist CGS 21680 (2*p*-(2-carboxyethylphen-ethylamino-5'-N-ethylcarboxamido)adenosine; 0.1–2.0 mg kg<sup>-1</sup> i.p.) exerted a striking improvement of dystonia.

**5** These data suggest that the precipitating effects of methylxanthines are, at least in part, related to their adenosine receptor antagonistic action.

**6** Although adenosine receptor agonists can be regarded as interesting candidates for the therapy of paroxysmal dystonia, adverse effects may limit the therapeutic potential of adenosine A<sub>1</sub> agonists, while beneficial effects of the adenosine A<sub>2A</sub> agonist CGS 21680 were already found at well tolerated doses.

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**Abbreviations:** CGS 15943, 9-chloro-2-(2-furyl)[1,2,4]triazolo[1,5-c]quinazolin-5-amine; CGS 21680, 2*p*-(2-carboxyethylphen-ethylamino-5'-N-ethylcarboxamido)adenosine; CPA, N<sup>6</sup>-cyclopentyladenosine; DMPX, 3,7-dimethyl-1-propargylxanthine; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; PDC, paroxysmal dystonic choreoathetosis; ZM 241385, 4-(2-[7-amino-2-(2-furyl)[1,2,4]triazolo[2,3-a][1,3,5]triazin-5-ylamino]ethyl)phenol

## Introduction

Dystonia is a movement disorder characterized by involuntary, sustained, patterned, and often repetitive muscle contractions of opposing muscles, frequently causing twisting movements or abnormal postures (Jankovic & Fahn, 1998). The pathophysiology of different types of idiopathic dystonia is not well understood, but there is evidence that basal ganglia dysfunctions can cause dystonic symptoms (Bhatia & Marsden, 1994; Fahn *et al.*, 1998). The existence of various phenotypic and genotypic subtypes indicates that the pathogenesis of idiopathic dystonias is heterogeneous (Fahn *et al.*, 1998). In a type of idiopathic paroxysmal dystonia (paroxysmal non-kinesiogenic dystonic choreoathetosis), attacks of generalized dystonia can be provoked by stress and by consumption of coffee or tea (Demirkiran & Jankovic,

1995; Fahn, 1994), i.e., by factors which are known to increase dopaminergic activity (Abercrombie *et al.*, 1989; Fredholm, 1995). However, the mechanism of the precipitating effects of the methylxanthines in paroxysmal dystonia has not yet been examined. Methylxanthines are known to inhibit adenosine A<sub>1</sub>/A<sub>2A</sub> receptors and, at higher concentrations, also cyclic nucleotide phosphodiesterases and the direct release of intracellular calcium (Fredholm, 1995). Blockade of adenosine receptors, which is known to interact with the dopaminergic activity in the striatum (see below), is possibly important for the induction of dystonic attacks by methylxanthines, since there is evidence that idiopathic dystonias are related to striatal dopaminergic dysfunctions (Todd & Perlmutter, 1998).

As shown in several previous studies, mutant dystonic hamsters (gene symbol *dt<sup>sz</sup>*), which show the phenotypic characteristics of this type of idiopathic paroxysmal dystonia in humans (Demirkiran & Jankovic, 1995; Richter &

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Löscher, 1998) are useful for preclinical drug testing and for giving insights into the underlying mechanism of this movement disorder. Previous studies have shown that striatal dopaminergic overactivity plays a crucial role in the manifestation of dystonic attacks in *dt<sup>sz</sup>* hamsters, probably based on GABAergic disinhibition (Gernert *et al.*, 2000; Rehders *et al.*, 2000; Richter & Löscher, 1998). Preliminary data indicated that caffeine aggravates dystonia in mutant dystonic hamsters (Richter & Löscher, 1998), allowing an examination of the mechanism of the precipitating effects of methylxanthines. This is important because knowledge about the mechanisms of the triggers of paroxysmal dystonia can contribute to our understanding of the pathophysiology of this movement disorder.

Adenosine, an endogenous nucleoside, modulates motor activity by interactions with the dopaminergic system within the basal ganglia (Fuxe *et al.*, 1998). Four different subtypes of G protein-coupled adenosine receptors have been described in the brain, namely A<sub>1</sub>, A<sub>2A</sub>, A<sub>2B</sub> and A<sub>3</sub> receptors, all of which have been cloned from several species (Fredholm *et al.*, 1994; Ongini & Fredholm, 1996). Specific antagonistic interactions between adenosine and dopamine receptors in the striatum are known between adenosine A<sub>1</sub> and dopamine D<sub>1</sub> receptors localized on GABAergic striatonigral/-entopeduncular neurons, and between adenosine A<sub>2A</sub> and dopamine D<sub>2</sub> receptors on GABAergic striatopallidal projections (Ferre *et al.*, 1997). Adenosine receptor antagonists, such as the methylxanthines, caffeine and theophylline, potentiate and adenosine receptor agonists inhibit the motor activating effects of dopamine receptor agonists (Ferre *et al.*, 1992).

Adenosine A<sub>2A</sub> antagonists exert anti-parkinsonian action (Kanda *et al.*, 1998; Müller, 2000), while adenosine receptor agonists have been suggested as new therapeutics for hyperkinetic basal ganglia disorders (Ferre *et al.*, 1997). However, examination of the therapeutic potential of adenosine receptor agonists for the treatment of hyperkinesias, such as paroxysmal dystonia, is still rare. Since permanent or stress-inducible paroxysmal dystonias/dyskinesias in humans are often intractable there is a need for new therapeutics. The observation that the intake of low doses of caffeine, which exerts adenosine antagonistic action, can provoke dystonic episodes, suggests that adenosine receptor agonists may exert beneficial effects also against stress-induced dystonia. Recently, first investigations of the effect of relatively high doses (0.5–2.0 mg kg<sup>-1</sup>) of the adenosine A<sub>2A</sub> receptor agonist CGS 21680 have shown beneficial effects in *dt<sup>sz</sup>* mutant hamsters (Richter *et al.*, 2000). Therefore, the therapeutic potential of the highly selective A<sub>1</sub> adenosine receptor antagonist CPA (Jacobson & Van Rhee, 1997) and of the highly selective A<sub>2A</sub> agonist CGS 21680 (Fredholm *et al.*, 1994; Ongini & Fredholm, 1996) for the treatment of paroxysmal dystonia was investigated in more detail in the present study. In order to examine whether the precipitating effects of methylxanthines may be related to their adenosine antagonistic action, the effects of the non-selective adenosine receptor antagonists caffeine and theophylline (Fredholm, 1995; Müller, 2000) on severity of dystonia were compared to those of selective adenosine receptor antagonists. CGS 15943 has high affinity for A<sub>2A</sub> receptors but it also potently blocks A<sub>1</sub> receptors (Ongini & Fredholm, 1996). The A<sub>1</sub> antagonist DPCPX displays about

700 fold selectivity for A<sub>1</sub> versus the A<sub>2A</sub> receptor (Jacobson & Van Rhee, 1997). DPMX, introduced as the first A<sub>2A</sub>-selective antagonist, also inhibits A<sub>2B</sub> receptors and at higher doses A<sub>1</sub> receptors, while ZM 241385 fulfill the criteria of a highly selective A<sub>2A</sub> antagonist (Müller, 2000; Ongini & Fredholm, 1996).

## Methods

### Animals

The present experiments were carried out in male and female *dt<sup>sz</sup>* mutant Syrian golden hamsters which were obtained by selective breeding as described in detail elsewhere (Fredow & Löscher, 1991). In mutant hamsters the motor disturbances are transmitted by a recessive gene (Löscher *et al.*, 1989). All dystonic hamsters were born and kept under the same controlled and constant environmental conditions. All experiments were done in compliance with the German Animal Welfare Act.

### Induction of dystonic attacks and severity-score of dystonia

As reported previously, motor impairments in *dt<sup>sz</sup>* hamsters show several features in common with human primary paroxysmal non-kinesigenic dystonia (paroxysmal dystonic choreoathetosis), characterized by long-lasting dystonic attacks (Demirkiran & Jankovic, 1995; Richter & Löscher, 1998). In mutant hamsters, dystonic attacks can be reproducibly induced by a triple stimulation technique (Löscher *et al.*, 1989; Richter & Löscher, 1998), i.e., stressful stimuli consisting of (1) taking the animal from its home cage and placing it on a balance, (2) injection of saline/vehicle (or of drugs), and (3) placement of the animal in a new plastic cage. After this procedure, *dt<sup>sz</sup>* hamsters develop a sequence of abnormal movements and postures. Therefore, the severity of dystonia can be rated by following score-system (Löscher *et al.*, 1989; Richter & Löscher, 1998): stage 1, flat body posture; stage 2, facial contortions, rearing with forelimbs crossing, 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, immobilization in a twisted, hunched posture with hind- and forelimbs tonically extended forward. After reaching the individual maximum stage the hamsters recover within 2–5 h. The individual maximum stage of dystonia is usually reached within 3 h after the hamsters were placed in the new cage. As described in several previous studies in detail (e.g., Richter & Löscher, 1993; 1998; Richter *et al.*, 2000), the dystonic syndrome in *dt<sup>sz</sup>* mutants shows an age-dependent time-course. The severity of dystonia reaches a maximum at an age of about 32–42 days. Thereafter, the severity slowly declines until complete remission occurs at an age of about 10 weeks. In the present study, all animals were examined for the presence of dystonia after weaning at the age of 21 days by the triple stimulation procedure three times per week until the animals exhibited constant individual severity scores and latencies to onset of unequivocal dystonic symptoms (stage 2). The present drug experiments were done during the life-

period of maximum expression of dystonia. Not all hamsters reach stage 6, but the individual maximum severity and the latency to onset is usually reproducible during this period (Richter & Löscher, 1998). To obtain reproducible latencies and avoid onset of dystonia prior or during the triple stimulation technique it was important to keep in time from taking the animals out of their home cage to placing them in a new cage (duration: 25–35 s). Animals that exhibited dystonic symptoms before injections of drug or vehicle were omitted from evaluation.

### Drug treatments

The effects of adenosine receptor agonists and antagonists on the severity of dystonia were examined in groups of 7–12 dystonic hamsters. Each group was used for one to two doses. In cases of repeated testing of drugs, the drug-free interval was 4 to 5 days. Dystonic attacks were induced by the procedure of triple stimulation, as described above. Since the individual maximum stage of dystonia (score rating system see above) is usually reached within 3 h, the hamsters were observed for 3 h after triple stimulation. For drug testing, a control trial was undertaken with the triple stimulation technique, injecting the vehicle used for drug administration (see below) by the same route of administration, i.e., i.p. or for control trial of theophylline s.c., and the latencies and severity of the dystonic attacks were noted after placing the animals in the new cage (pre-drug control). Two days later, the drug was administered in the same group of animals and the latency and severity were noted. Furthermore, animals were observed for central adverse effects. As described for pre-drug-controls, a control trial with vehicle was done 2 days after drug treatment (post-drug control). Hamsters that differed in the maximum severity of dystonia in the pre-drug and post-drug control trials by more than two stages (about 4%) were omitted from the drug evaluation. All control and drug trials were done at the same time of the day between 0900 and 1200 h.

The examiner rating the severity of dystonia was blind to the treatment condition of the animals or in cases of unequivocal side effects at least unaware of the drugs used in the present experiments. Apart from examination of the effects of adenosine receptor agonists and antagonists on dystonia, the side effects (in part not quantified) were observed by a second examiner in order to assure that sufficient doses, i.e., central acting doses, were chosen (see below). Animal locomotor activity was determined by a score system, as used in previous studies (e.g., Richter & Löscher, 1995). In cases of a marked reduction of locomotor activity after drug administration, the catalepsy response was determined by quantifying descent latency (in seconds) in a block test in comparison to the post-drug vehicle control. The hamsters were placed with the forelimbs on a block (6 cm high) and the descent latency, i.e., the time over which the animals maintained this position, was noted. The effects of adenosine receptor agonists on body temperature were determined by rectal measurements in separate groups of 5–6 *dt<sup>sz</sup>* hamsters.

### Drugs

The non-selective adenosine receptor antagonists caffeine and theophylline (Sigma, Deisenhofen, Germany) were dissolved

in isotonic saline. The adenosine  $A_{1/2A}$  receptor antagonist 9-chloro-2-(2-furyl) [1,2,4]triazolo [1,5-c]quinazolin-5-amine (CGS 15943), kindly provided by Novartis (Summit, NJ, U.S.A.), the  $A_1$  receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX; Biotrend, Cologne, Germany) and the  $A_{2A}$  receptor antagonist 4-(2-[7-amino-2-(2-furyl)][1,2,4]triazolo[2,3-a][1,3,5]triazin-5-ylamino)ethylphenol (ZM 241385; Biotrend, Cologne, Germany) were suspended in 0.3% Tween 80. The adenosine  $A_2$  receptor antagonist 3,7-dimethyl-1-propargylxanthine (DMPX; Biotrend, Cologne, Germany) was dissolved in water. The adenosine  $A_1$  agonist  $N^6$ -cyclopentyladenosine (CPA, Biotrend, Cologne, Germany) and the adenosine  $A_{2A}$  agonist 2*p*-(2-carboxyethylphenethylamino-5'-N-ethylcarboxamino)adenosine (CGS 21680; Biotrend, Cologne, Germany) were dissolved in 0.3% Tween 80. All solutions and suspensions were freshly prepared. For all drug administrations, injection volume was 5 ml kg<sup>-1</sup>. For pre- and post-drug control recordings the animals received the same volume of vehicle.

The doses of these drugs and routes of administration were chosen on the basis of several previous experiments in which central effects of adenosine receptor ligands were investigated in rodents (e.g., Klitgaard *et al.*, 1993; Pinna *et al.*, 1996; Yacoubi *et al.*, 2000). The doses of these drugs were increased until unequivocal behavioural effects could be observed in mutant hamsters. This criterion was fulfilled for all compounds at least for the highest doses. All drugs were injected intraperitoneally. In addition, theophylline was administered subcutaneously in order to obtain a longer duration of action than observed after i.p. injections.

### Statistics

The significance of differences (severity of dystonia, latencies to onset of dystonia, adverse effects) between control trials (pre- and post-drug) and drug trial in the same group of animals was calculated by the Friedman test and, if a significant difference (at least  $P < 0.05$ ) was found, the Wilcoxon signed rank test for paired replicates was used post hoc to determine which pairs differed. The significance of differences between different groups (i.e. effects of adenosine receptor agonists on body temperature vs vehicle, effects on dystonia of caffeine or adenosine receptor agonists administered alone vs co-administrations) was evaluated by the Kruskal–Wallis test and, if a significant difference (at least  $P < 0.05$ ) was found, the Mann–Whitney U-test was used to analyse which groups differed.

## Results

### Effects of adenosine receptor antagonists

*Methylxanthines (non-selective adenosine  $A_1/A_{2A}$  receptor antagonists)* In *dt<sup>sz</sup>* hamsters, caffeine significantly accelerated the progression of dystonia at doses of 10 and 20 mg kg<sup>-1</sup> i.p., i.e., the severity of dystonia was significantly increased during the first hour of observation (Figure 1) and the latency to onset of dystonia was significantly decreased (Table 1). Furthermore, the latency to stage 6 was significantly reduced to  $60.0 \pm 6.8$  min vs  $128.0 \pm 18.2$  min (pre-drug control,  $P < 0.01$ ) or  $122.5 \pm 18.7$  min (post-drug

control,  $P < 0.05$ ) after administration of  $10 \text{ mg kg}^{-1}$  i.p. and to  $29.6 \pm 4.5 \text{ min}$  vs  $102.2 \pm 18.6 \text{ min}$  (pre-drug control,  $P < 0.05$ ) or  $120.2 \pm 9.2 \text{ min}$  (post-drug control,  $P < 0.05$ ) after i.p. injection of  $20 \text{ mg kg}^{-1}$ . At a lower dose of  $5 \text{ mg kg}^{-1}$  i.p. (not illustrated) caffeine did not exert any prodystonic effects. Behavioural effects were moderate hyperlocomotion during the first hour after administration of  $20 \text{ mg kg}^{-1}$  i.p., while no side effects could be observed at the lower doses.

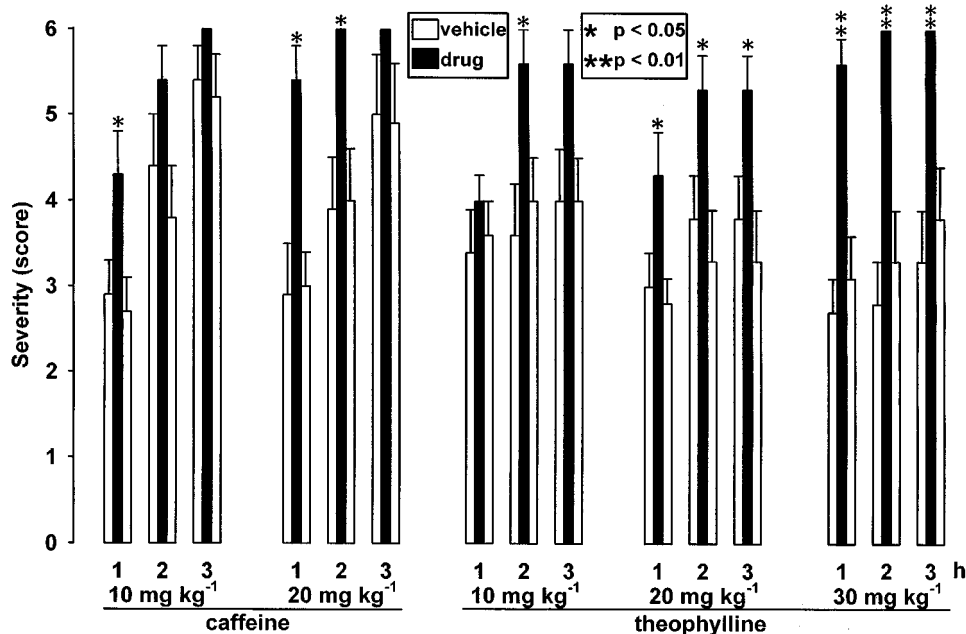
Theophylline caused a marked aggravation of dystonia after subcutaneous injections of 10, 20 and  $30 \text{ mg kg}^{-1}$  (Figure 1), but not at a dose of  $5 \text{ mg kg}^{-1}$  (not illustrated). By using animal groups with lower expression of dystonia (see Figure 1, pre- and post-drug control data) than those used for the experiments with caffeine, not only an acceleration of the progression of dystonia, but also a significant increase of the maximum severity reached during the 3-h observation period became evident after treatment with 20 and  $30 \text{ mg kg}^{-1}$  s.c. As shown in Table 1, theophylline significantly decreased the latency to onset of dystonia. Intraperitoneal injections of  $20 \text{ mg kg}^{-1}$  of theophylline exerted only short-lasting effects, but accelerated the progression of dystonia, i.e., a significant increase of the severity was observed during the first hour after administration (not illustrated) and the latency to onset of dystonia was significantly reduced. At low doses of 5 and  $10 \text{ mg kg}^{-1}$  s.c. theophylline did not cause any adverse effects. Twenty  $\text{mg kg}^{-1}$  s.c. moderately increased the locomotor activity only during the first 20 min after administration, while

$30 \text{ mg kg}^{-1}$  s.c. caused an increased urinary output and more marked hyperlocomotion, which lasted up to 60 min after injection.

*The selective adenosine  $A_{1/2A}$  receptor antagonist* CGS 15943 significantly increased the individual maximum severity of dystonia (i.e., the severity reached during the third hour of observation) at a dose of  $30 \text{ mg kg}^{-1}$  i.p., but not at a lower dose of  $20 \text{ mg kg}^{-1}$  (Figure 2). At both tested doses, CGS 15943 tended to increase the severity also during the first 2 h after administration. CGS 15943 did not reduce the latency to onset of dystonia (Table 1), probably because of a delayed onset of action as indicated by the relatively late occurrence of behavioural effects. About 15 min after i.p. administration of 20 or  $30 \text{ mg kg}^{-1}$  the hamsters showed a moderate hyperactivity which lasted for about 60 min.

*The selective adenosine  $A_1$  receptor antagonist* DPCPX significantly increased the severity of dystonia at doses of 10 and  $20 \text{ mg kg}^{-1}$  (Figure 3). The latency to onset of dystonia was significantly reduced after administration of  $20 \text{ mg kg}^{-1}$  i.p. DPCPX (Table 1). The hamsters exhibited a moderate hyperactivity 10–40 min after i.p. injection of  $10 \text{ mg kg}^{-1}$ . At a dose of  $20 \text{ mg kg}^{-1}$  i.p. a more marked hyperlocomotion was observed already after 3 min and lasted for about 1 h.

In contrast to DPCPX, the adenosine  $A_2$  receptor antagonist DMPX at a dose of 2 mg i.p. (Figure 3) or at a higher dose of  $4 \text{ mg kg}^{-1}$  i.p. (not illustrated) and the



**Figure 1** Effects of the methylxanthines caffeine ( $10.0$  and  $20.0 \text{ mg kg}^{-1}$  i.p.) and theophylline ( $10.0$ ,  $20.0$  and  $30.0 \text{ mg kg}^{-1}$  s.c.) on severity of dystonia in mutant hamsters. Usually, the individual maximum severity of dystonia is reached within 3 h after induction of dystonia by triple stimulation including the injection of drugs (black 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 first, second and third hour after administration of the methylxanthine or vehicle, reflecting the progression of dystonia in  $dt^{sz}$  hamsters after treatment with the active compound and during 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 aggravation of dystonia in comparison to the pre- and post-drug control (\* $P < 0.05$ , \*\* $P < 0.01$ ). Data are shown as means  $\pm$  s.e. of seven ( $20 \text{ mg kg}^{-1}$  caffeine), eight ( $10$  and  $20 \text{ mg kg}^{-1}$  theophylline), nine ( $30 \text{ mg kg}^{-1}$  theophylline) or 10 ( $10 \text{ mg kg}^{-1}$  caffeine) dystonic hamsters. Absence of s.e. bars indicates that all hamsters had reached the same severity.

**Table 1** Drug effects on latency to onset of dystonia in *dt<sup>sz</sup>* mutant hamsters

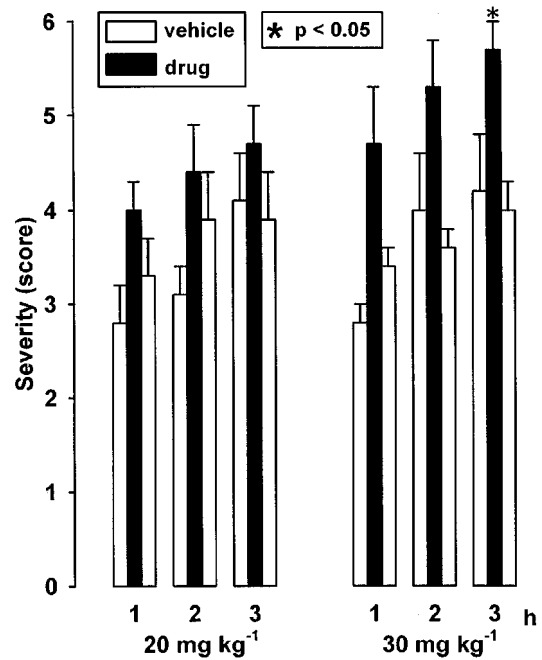
Dose (mg kg <sup>-1</sup> )	Latency (min)			Post-drug (n)
	Pre-drug	Drug	Post-drug	
<b>Caffeine</b>				
10.0	5.1 ± 0.5	3.6 ± 0.5 <sup>a</sup>	7.0 ± 1.3	10
20.0	9.9 ± 0.9	5.0 ± 0.4 <sup>b</sup>	9.1 ± 2.1	7
<b>Theophylline</b>				
10.0	6.9 ± 0.9	4.9 ± 0.6 <sup>a</sup>	7.4 ± 1.6	8
20.0	12.3 ± 1.3	5.5 ± 0.7 <sup>b</sup>	9.2 ± 1.3	8
30.0	12.8 ± 1.2	6.4 ± 0.8 <sup>b</sup>	10.0 ± 1.7	9
<b>CGS 15943</b>				
20.0	9.6 ± 1.4	9.3 ± 1.0	7.6 ± 0.6	11
30.0	7.7 ± 1.0	9.9 ± 1.3	10.6 ± 1.0	9
<b>DPCPX</b>				
10.0	11.6 ± 1.7	8.3 ± 1.0	14.4 ± 1.8	9
20.0	15.7 ± 2.3	4.8 ± 0.6 <sup>b</sup>	8.8 ± 1.3	10
<b>DMPX</b>				
2.0	7.9 ± 1.0	6.0 ± 0.8	7.9 ± 1.0	10
4.0	9.0 ± 1.0	8.7 ± 0.4	6.4 ± 0.8	7
<b>ZM 241385</b>				
2.0	7.7 ± 1.5	9.4 ± 1.4	13.9 ± 1.3	7
5.0	13.3 ± 2.1	12.2 ± 2.2	10.3 ± 1.4	7
<b>CPA</b>				
0.05	8.7 ± 0.6	15.2 ± 5.4	11.6 ± 1.1	12
0.1	5.3 ± 1.9	20.7 ± 6.0 <sup>b</sup>	6.7 ± 1.2	9
0.25	7.1 ± 2.3	68.6 ± 16.7 <sup>b</sup>	5.7 ± 1.3	7
0.5	n.d.	n.d.	n.d.	7
1.0	n.d.	n.d.	n.d.	7
<b>CGS 21680</b>				
0.1	7.6 ± 1.2	37.7 ± 10.2 <sup>b</sup>	9.3 ± 1.0	10
0.25	6.9 ± 1.4	103.9 ± 5.1 <sup>b</sup>	5.1 ± 0.5	10
0.5	7.0 ± 0.8	85.0 ± 8.0 <sup>a</sup>	8.0 ± 0.8	7
1.0	8.0 ± 1.1	134.4 ± 10.3 <sup>b</sup>	8.0 ± 1.1	9
2.0	5.5 ± 2.9	148.0 ± 6.9 <sup>c</sup>	8.5 ± 2.1	8

Latency was determined as the time to the first unequivocal signs of the systonic attacks (stage 2). Data are shown as means ± s.e. mean of the number of animals indicated (*n*). Significant differences to pre-drug and post-drug controls are marked by <sup>a</sup>(*P* < 0.05) and <sup>b</sup>(*P* < 0.01). <sup>c</sup>Not determined to be significant because less than five animals reached stage 2 after drug administration. Since CPA caused severe side effects at higher doses, stage 2 could not be determined (n.d.).

selective A<sub>2A</sub> receptor antagonist ZM 241385 at doses of 2 (not illustrated) and 5 mg kg<sup>-1</sup> i.p. (Figure 3) failed to exert any effects on the severity of dystonia and on latency to onset (Table 1). Both compounds caused a moderate hyperlocomotion at the tested doses during the first 30–60 min after administration.

#### Effects of adenosine receptor agonists

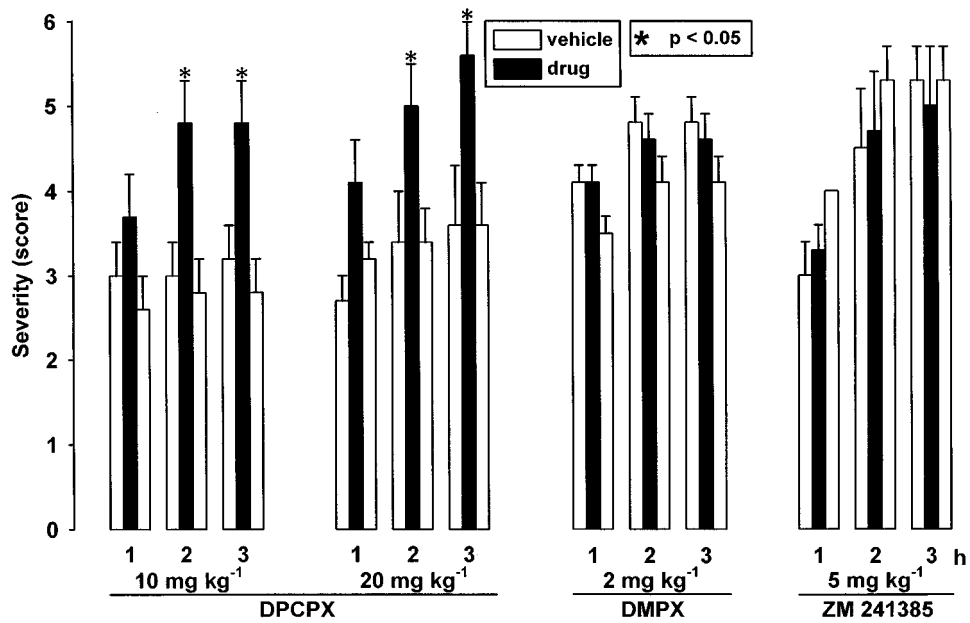
As shown in Figure 4, the adenosine A<sub>1</sub> receptor agonist CPA exerted enormous antidystonic effects. At a dose of 1.0 mg kg<sup>-1</sup> i.p., CPA completely prevented the occurrence of dystonia during the first 2 h after the induction of an attack by the triple stimulation. A delayed progression of dystonia and a dose-dependent decrease of the individual maximum severity of dystonia, reached at the end of the 3-h observation period, was also observed after i.p. administration of 0.1, 0.25 and 0.5 mg kg<sup>-1</sup> (Figure 4), while 0.05 mg kg<sup>-1</sup> did not exert significant effects on the severity or latency to onset of dystonia (not illustrated). A significant increase of the latency to onset of dystonia could be observed



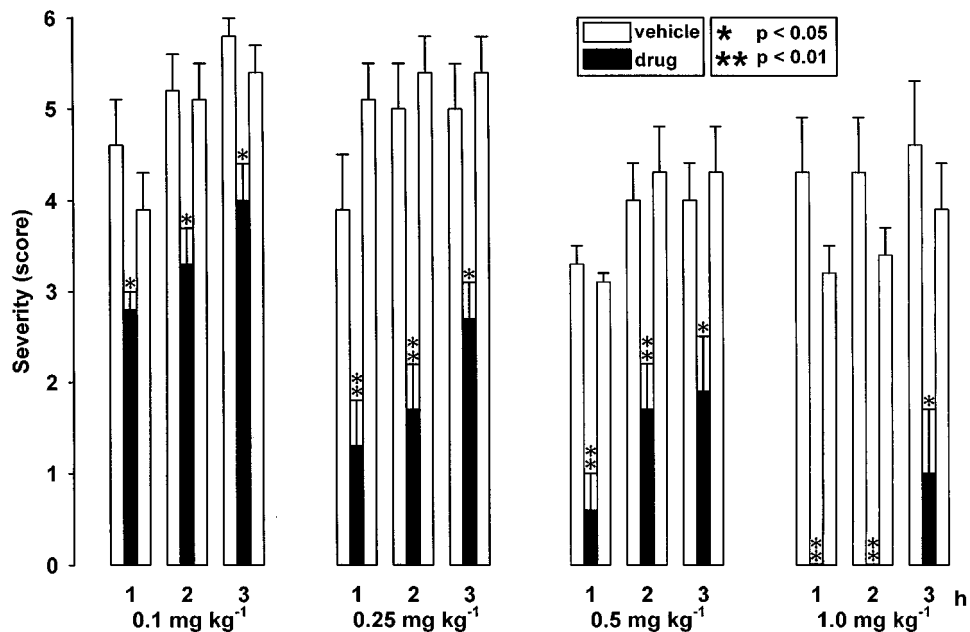
**Figure 2** Effect of the adenosine A<sub>1/2A</sub> receptor antagonist CGS 15943 on severity of dystonia in mutant hamsters after administration of 20 and 30 mg kg<sup>-1</sup> i.p. The figure shows the average of the maximum individual severity scores of dystonia reached within the first, second and third hour after administration of CGS 15943. Control recordings were taken 2 days before (pre-drug control) and 2 days after (post-drug control) the drug trial. Asterisks indicate significant aggravation of dystonia in comparison to the pre- and post-drug control (\**P* < 0.05). Data are shown as means ± s.e. of 11 (20 mg kg<sup>-1</sup>) or nine (30 mg kg<sup>-1</sup>) hamsters. For further explanation see Figure 1 legend.

at doses of 0.1 and 0.25 mg kg<sup>-1</sup> i.p. (Table 1), while central side effects exerted at higher doses disturbed the evaluation of stage 2. Striking antidystonic effects after treatment with 1.0 mg kg<sup>-1</sup> i.p. were accompanied by severe adverse effects, i.e., a marked ataxia, sedation, piloerection and a loss of spontaneous locomotor activity. However, 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 reveal any catalepsy. The reduced locomotor activity observed 5 min after treatment lasted for about 2 h, followed by short periods of head tremor. Ataxia and hypolocomotion were also intensive after i.p. injections of 0.5 mg kg<sup>-1</sup> CPA. At a dose of 0.25 mg kg<sup>-1</sup> i.p., the depressant effect on motor activity was still present and the animals showed a moderate ataxia within the first 2 h, while no behavioural effects could be observed at lower doses of 0.1 and 0.05 mg kg<sup>-1</sup> i.p.

The adenosine A<sub>2A</sub> receptor agonist CGS 21680 significantly decreased the severity of dystonia (Figure 5) and increased the latency to onset (Table 1) not only at the higher doses of 0.5–2 mg kg<sup>-1</sup> i.p., as shown by recent examinations (Richter *et al.*, 2000). Significant antidystonic effects could be already observed at doses of 0.1 mg kg<sup>-1</sup> i.p., which did not provoke any observable side effects, and of 0.25 mg kg<sup>-1</sup> i.p. which caused a moderate reduction of the spontaneous activity 5–70 min after administration. As indicated by the early disappearance of adverse effects, the duration of action of CGS 21680 was shorter than this of



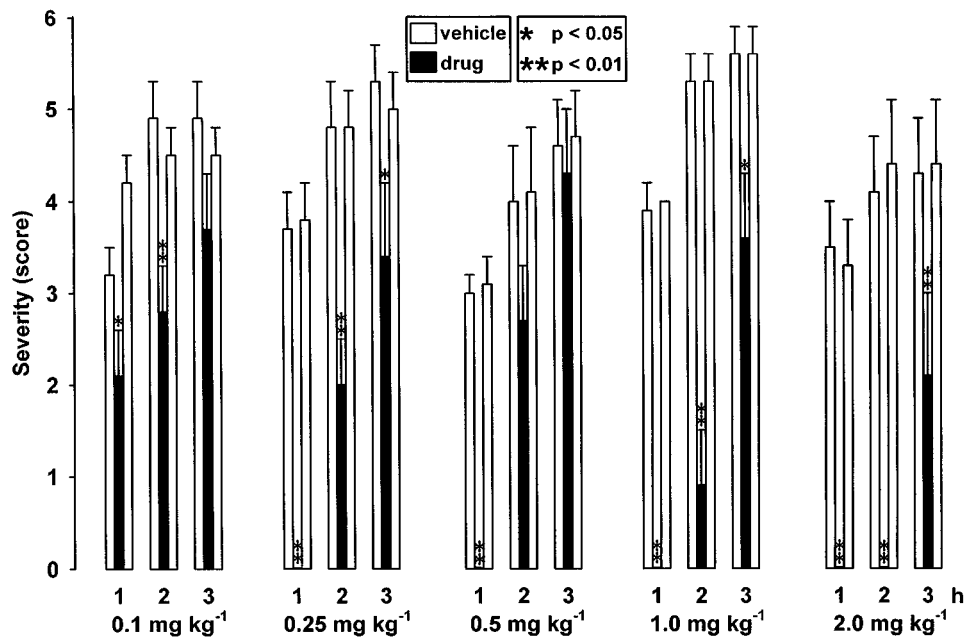
**Figure 3** Effect of the adenosine A<sub>1</sub> receptor antagonist DPCPX (10 and 20 mg kg<sup>-1</sup> i.p.), of the A<sub>2</sub> receptor antagonist DMPX (2 mg kg<sup>-1</sup> i.p.) and of the A<sub>2A</sub> antagonist ZM 241385 (5 mg kg<sup>-1</sup> i.p.) on severity of dystonia in mutant hamsters. The figure shows the average of the maximum individual severity scores of dystonia reached within the first, second and third hour after drug administration. Control recordings were taken 2 days before (pre-drug control) and 2 days after (post-drug control) the drug trial. Asterisks indicate significant aggravation of dystonia in comparison to the pre- and post-drug control (\**P* < 0.05). Data are shown as means ± s.e. of seven (ZM 241385), nine (10 mg kg<sup>-1</sup> DPCPX) or 10 (2 mg kg<sup>-1</sup> DMPX, 20 mg kg<sup>-1</sup> DPCPX) mutant hamsters. For further explanation see Figure 1 legend.



**Figure 4** Effect of the adenosine A<sub>1</sub> receptor agonist CPA on severity of dystonia in mutant hamsters after administration of 0.1, 0.25, 0.5 and 1.0 mg kg<sup>-1</sup> i.p. The figure shows the average of the maximum individual severity scores of dystonia reached within the first, second and third hour after administration of CPA. Control recordings were taken 2 days before (pre-drug control) and 2 days after (post-drug control) the drug trial. Asterisks indicate significant reduction 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 (0.25–1.0 mg kg<sup>-1</sup>) or nine (0.1 mg kg<sup>-1</sup>) *dr<sup>z</sup>* hamsters. For further explanation see Figure 1.

CPA at a same dose of 0.25 mg kg<sup>-1</sup> i.p. Even at a high dose of 2.0 mg kg<sup>-1</sup> i.p., CGS 21680 exerted less marked adverse effects than CPA at doses of 0.5 and 1 mg kg<sup>-1</sup> i.p.

As determined in an additional group of five mutant hamsters, CGS 21680 and CPA decreased the rectal body temperature at a dose of 0.5 mg kg<sup>-1</sup> i.p. in comparison to



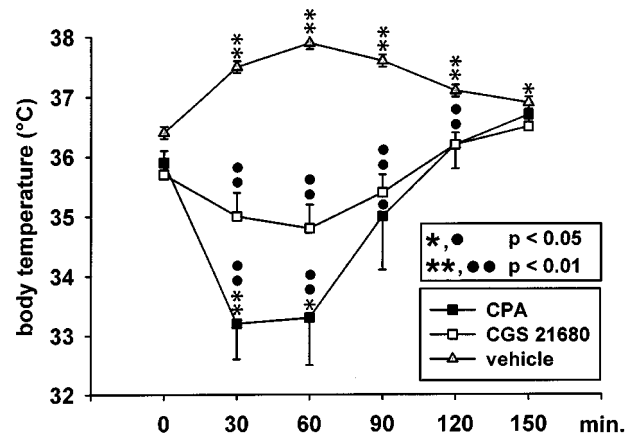
**Figure 5** Effect of the adenosine  $A_{2A}$  receptor agonist CGS 21680 on severity of dystonia in mutant hamsters after administration of 0.1, 0.25, 0.5, 1.0 and 2.0  $\text{mg kg}^{-1}$  i.p. Note that CGS 21680 already exerts antidystonic effects at lower doses of 0.1 and 0.25  $\text{mg kg}^{-1}$  than at those described recently (0.5–2.0  $\text{mg kg}^{-1}$ ; Richter *et al.*, 2000). The figure shows the average of the maximum individual severity scores of dystonia reached within the first, second and third hour after administration of CGS 21680. Control recordings were taken 2 days before (pre-drug control) and 2 days after (post-drug control) the drug trial. Asterisks indicate a significant reduction of dystonia in comparison to the pre- and post-drug control (\* $P < 0.05$ ; \*\* $P < 0.01$ ). Data are shown as means  $\pm$  s.e. of eight (2  $\text{mg kg}^{-1}$ ), nine (1  $\text{mg kg}^{-1}$ ) or 10 (0.1, 0.25  $\text{mg kg}^{-1}$ ) hamsters. For further explanation see Figure 1 legend.

the vehicle control group (Figure 6). CPA, but not CGS 21680, caused a significant hypothermia 30 and 60 min after administration in comparison to animals of the same group prior treatment, while the body temperature increased after injection of the vehicle Tween 80, probably caused by an enhanced muscle activity during dystonic attacks.

As shown in Figure 7, i.p. injections of antidystonic effective doses (0.25 and 0.5  $\text{mg kg}^{-1}$ ) of the  $A_1$  receptor agonist CPA 10 min prior administration of 20  $\text{mg kg}^{-1}$  i.p. caffeine could counteract the prodystonic effects of caffeine. Otherwise, caffeine significantly reduced the antidystonic efficacy of CPA (compare Figure 4), obtained after single administrations of 0.25  $\text{mg kg}^{-1}$  i.p. (first to second hour,  $P < 0.01$ ) and of 0.5  $\text{mg kg}^{-1}$  i.p. (first hour not significant; second hour  $P < 0.01$ ; third hour  $P < 0.05$ ). Pretreatments with the  $A_{2A}$  receptor agonist CGS 21680 at doses of 0.25 and 0.5  $\text{mg kg}^{-1}$  i.p. failed to prevent the caffeine-provoked aggravation of dystonia in mutant hamsters (Figure 7). The antidystonic efficacy of CGS 21680, observed after single administrations (see Figure 5), completely disappeared after treatment with caffeine ( $P < 0.01$  vs single injections of 0.25 or 0.5  $\text{mg kg}^{-1}$  i.p. during the first and second hour;  $P < 0.05$  vs both doses during the third hour after single administrations).

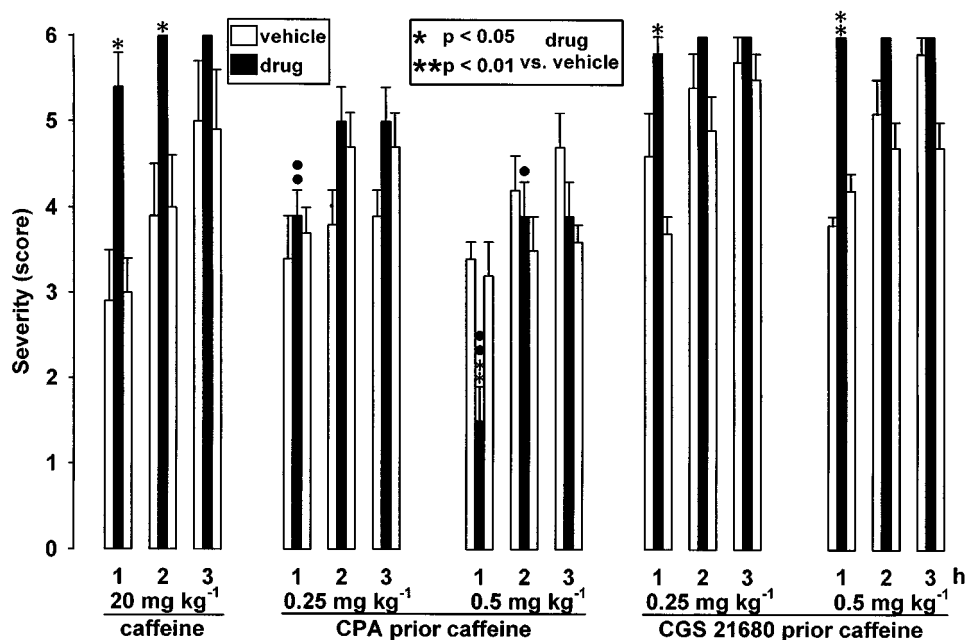
## Discussion

The present data showing significant prodystonic effects of the methylxanthines caffeine and theophylline substantiate the  $dr^{sz}$  mutant hamster as a valid animal model of hereditary paroxysmal dystonic choreoathetosis (PDC) because inges-



**Figure 6** Effect of the adenosine  $A_1$  receptor agonist CPA (0.5  $\text{mg kg}^{-1}$  i.p.) and of the adenosine  $A_{2A}$  receptor agonist CGS 21680 (0.5  $\text{mg kg}^{-1}$  i.p.) on rectal temperature in a group of  $dr^{sz}$  mutant hamsters. The figure shows the average body temperature prior (0 min) and after drug administrations (30–150 min) in comparison to a control group treated with the vehicle (Tween 80). Data are shown as means  $\pm$  s.e. of five (CPA and CGS 21680) or six (vehicle control) mutant hamsters. Asterisks indicate significant differences after injections of vehicle or CPA versus prior treatment in the same group of animals (\* $P < 0.05$ , \*\* $P < 0.01$ ). Circles indicate a significant decrease in comparison to the vehicle control group (\* $P < 0.05$ ; \*\* $P < 0.01$ ).

tion of coffee and tea are (apart from stress and exercise) the most common precipitating factors of dystonic episodes in patients (Demirkiran & Jankovic, 1995; Fahn, 1994). Caffeine and theophylline worsened dystonia in  $dr^{sz}$  hamsters already at doses which did not provoke hyperlocomotion, i.e., the



**Figure 7** Pretreatments with the adenosine  $A_{2A}$  receptor agonist CGS 21680 (0.25 mg and 0.5  $\text{mg kg}^{-1}$  i.p.) or with the  $A_1$  receptor agonist CPA (0.25 and 0.5  $\text{mg kg}^{-1}$  i.p.) 10 min prior i.p. injections of 20  $\text{mg kg}^{-1}$  caffeine. The figure shows the average of the maximum individual severity scores of dystonia reached within the first, second and third hour after caffeine alone and after pretreatments. Asterisks indicate significant differences of the severity of dystonia in comparison to the pre- and post-drug control ( $*P < 0.05$ ). Circles indicate a significant reduction of the differences of severity score (caffeine vs control data) in animal groups pretreated with CPA ( $*P < 0.05$ ;  $**P < 0.01$ ). Data are shown as means  $\pm$  s.e. of seven (20  $\text{mg kg}^{-1}$  caffeine), nine (caffeine after pretreatment with 0.5  $\text{mg kg}^{-1}$  CPA or CGS 21680) or 10 (caffeine after 0.25  $\text{mg kg}^{-1}$  CPA or CGS 21680) mutant hamsters. For further explanation see Figure 1 legend.

prodystonic effects are not merely due to an increase in locomotor activity. Considering a shorter half-life time of methylxanthines in rodents than in man (Fredholm, 1995), prodystonic effective levels in  $dt^{sz}$  hamsters are probably comparable to those reached after coffee intake in humans. Matsuo *et al.* (1999) reported that patients with PDC developed severe attacks within 40 min after oral intake of 300 mg caffeine, an amount known to be contained in about three cups of coffee.

Methylxanthines inhibit adenosine  $A_1$  and  $A_{2A}$  receptors at low concentrations, while inhibition of phosphodiesterase and of intracellular calcium release occurs at much higher brain levels than those attained by usual caffeine consumption (Fredholm, 1995). In fact, the prodystonic effects of the selective adenosine  $A_1/A_{2A}$  receptor antagonist CGS 15943 and of the  $A_1$  antagonist DPCPX found in this study indicate that the induction of dystonic episodes by methylxanthines is, at least in part, related to their adenosine receptor antagonist action. The prodystonic effects of caffeine and theophylline were, however, more pronounced than those of CGS 15943, an antagonist with a higher affinity for  $A_{2A}$  than  $A_1$  receptors (Ongini & Fredholm, 1996) and  $A_{2/2A}$  receptor antagonists failed to exert any effects on the severity of dystonia in  $dt^{sz}$  hamsters. In view of the behavioural effects caused by both  $A_1$  and  $A_2$  antagonists, it seems unlikely that the lack of prodystonic effects of  $A_2$  receptor inhibitors is related to insufficient penetration of these drugs into the brain. Therefore, the precipitating effects of methylxanthines seem to be predominantly mediated by an adenosine  $A_1$  receptor blockade. This is substantiated by the finding that pretreatment with the  $A_1$  receptor agonist CPA, but not with the  $A_{2A}$

agonist CGS 21680, counteracted the prodystonic effect of caffeine.

Several studies have shown that methylxanthines and inhibitors of  $A_1$  receptors increase striatal dopamine release, while  $A_{2A}$  receptor blockers do not cause any changes in extracellular dopamine levels in the rodent striatum (Ferre *et al.*, 1993; Okada *et al.*, 1997; Zetterström & Fillenz, 1990). There is strong evidence that striatal dopaminergic overactivity plays an important role in the manifestation of paroxysmal dystonia in mutant hamsters (Nobrega *et al.*, 1996; Rehders *et al.*, 2000). Thus, a further increase in dopamine release in the striatum provoked by  $A_1$  receptor blockade, is probably relevant for the prodystonic effects of methylxanthines and DPCPX in  $dt^{sz}$  mutants. Ongoing microdialysis studies in mutant hamsters have to clarify whether the aggravation of dystonia caused by caffeine is accompanied by a rise of extracellular dopamine levels.

Recent investigations suggested that the striato-entopeduncular activity is enhanced in  $dt^{sz}$  mutants (Gernert *et al.*, 1999; 2000). The activity of striato-entopeduncular neurons is regulated *via* interacting adenosine  $A_1$  and  $D_1$  receptors (Ferre *et al.*, 1996; 1997). A further increase of striato-entopeduncular activity by  $A_1$  receptor blockers can therefore also be important for the prodystonic effects of methylxanthines and DPCPX in dystonic hamsters. In contrast to  $A_1$  receptor blockade, adenosine  $A_1$  receptor agonists are known to reduce striatal dopamine release and striato-entopeduncular activity (Ferre *et al.*, 1996; Okada *et al.*, 1997), which may explain the striking antidystonic efficacy of CPA in  $dt^{sz}$  mutants.



Apart from methylxanthines, known as triggers of dystonic attacks in patients with PDC (Demirkiran & Jankovic, 1995; Fahn, 1994), the present data suggest that antagonists of A<sub>1</sub> receptors, but not of A<sub>2</sub> receptors, may carry a risk of inducing dyskinetic episodes in predisposed individuals. Interestingly, adenosine A<sub>2A</sub> receptor antagonists have been reported to exert anti-parkinsonian efficacy in parkinsonian monkeys without leading to dyskinesias (Kanda *et al.*, 1998). This observation is in line with the present finding that A<sub>2A</sub> receptor inhibitors did not worsen paroxysmal dyskinesia in mutant hamsters.

Stimulation of A<sub>2A</sub> receptors, which are co-localized with D<sub>2</sub> receptors on striatopallidal neurons, completely counteracts a D<sub>2</sub> receptor agonist-induced inhibition of these GABAergic projection neurons (Ferre *et al.*, 1997). This mechanism may provide an explanation for the pronounced beneficial effects of the A<sub>2A</sub> receptor agonist CGS 21680 in dystonic hamsters, because previous intrastriatal injections of the dopamine D<sub>2</sub> receptor agonist quinpirole worsened dystonia in *dr<sup>sz</sup>* hamsters (Rehders *et al.*, 2000). However, adenosine also modulates other neurotransmitters, such as GABA release from interneurons, which is increased by A<sub>2A</sub> receptor agonists (Sebastiao & Ribeiro, 2000). Since dopaminergic overactivity is probably secondary to an inborn deficit of striatal GABAergic interneurons in *dr<sup>sz</sup>* hamsters (Gernert *et al.*, 2000), the antidystonic efficacy of CGS 21680 could also be related to increased GABA release. Thus, further striatal microinjections of adenosine and dopamine receptor ligands in mutant hamsters have to clarify if the present findings with adenosine receptor agonists and antagonists are related to their interaction with the dopaminergic system.

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. Paroxysmal dyskinesias are usually worsened by L-dopa, while benzodiazepines and neuroleptics exert beneficial effects in PDC (Fahn, 1994; Przuntek & Monninger, 1983). Fink *et al.* (1997) proposed that dystonic episodes occur during a period of striatal dopamine depletion after caffeine-induced dopamine release. Methylxanthines provoke an increase in extracellular dopamine levels in the rat striatum within 1 h of administration (Okada *et al.*, 1997). The fast onset of prodystonic effects of methylxanthines in mutant hamsters, shown in the

present study, and the rapid occurrence of dystonic episodes in patients with PDC after oral caffeine intake (Matsuo *et al.*, 1999), suggest that dopaminergic overactivity is essential for the manifestation of this type of paroxysmal dystonia.

The striking antidystonic efficacy of CPA and CGS21680, demonstrated in the present study, suggests that adenosine A<sub>1</sub> and A<sub>2A</sub> receptor agonists may provide new approaches for the treatment of paroxysmal dystonia, but adverse effects may limit their therapeutic use. As observed in the present study in *dr<sup>sz</sup>* hamsters, both the A<sub>1</sub> agonist CPA and the A<sub>2A</sub> agonist CGS 21680 exert hypothermia and depressant effects on locomotor activity similar to neuroleptics (Fuxe *et al.*, 1998; Richter & Löscher, 1993). Hypotensive effects due to vasodilatation can be provoked by A<sub>1</sub> and A<sub>2A</sub> agonists as well as by neuroleptics (Nyce, 1999; Ongini & Fredholm, 1996). While long-term treatment with neuroleptics, however, is associated with a risk of tardive dyskinesia (Marsden & Quinn, 1990), adenosine A<sub>2A</sub> receptor agonists may attenuate tardive dyskinesias (Fuxe *et al.*, 1998). Therefore, in types of dystonia in which neuroleptics exert beneficial effects, such as in paroxysmal dystonia (Fahn, 1994; Richter & Löscher, 1993), adenosine A<sub>2A</sub> receptor agonists may prove advantageous. Furthermore, in *dr<sup>sz</sup>* hamsters the antidystonic efficacy of CGS21680 was more marked than that of neuroleptics (Richter & Löscher, 1993). In contrast to CPA, which is also known to cause a depression of cardiac function and bronchoconstriction (Nyce, 1999), CGS 21680 exerted marked antidystonic action not only at high doses, as shown in a recent study in *dr<sup>sz</sup>* mutants (Richter *et al.*, 2000), but also at well tolerated doses.

In summary, the present data indicates that induction of dystonic attacks by methylxanthines in PDC is, at least in part, related to their adenosine antagonistic effects. The pronounced antidystonic efficacy of adenosine receptor agonists in *dr<sup>sz</sup>* mutants indicates that these compounds could provide new approaches for the treatment of hyperkinetic basal ganglia disorders. In view of the antidystonic efficacy of CGS 21680 at well tolerated doses, particularly adenosine A<sub>2A</sub> receptor agonists are interesting candidates for the treatment of paroxysmal dystonia.

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