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Catalepsy induced by a blockade of dopamine D_1 or D_2 receptors was reversed by a concomitant blockade of adenosine A_{2A} receptors in the caudate-putamen of rats

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Abstract

The present study sought to determine, in more detail, the effects of an unselective and a selective adenosine A_{2A} receptor blockade on catalepsy induced by a blockade of dopamine D_1 or D_2 receptors in rats. The results demonstrated that systemic administration of the unselective A_1/A_2 receptor antagonist, theophylline and the selective A_{2A} receptor antagonist, CSC potently reversed catalepsy induced by a systemic D_2 receptor blockade with raclopride or by a bilateral blockade of D_2 receptors in the caudate-putamen (CPu) with S(–)sulpiride. Likewise, systemic administration of theophylline and CSC reversed catalepsy induced by a systemic D_1 receptor blockade with SCH23390; theophylline also counteracted catalepsy after an intra-CPu D_1 receptor blockade with SCH23390. Intracerebral co-microinfusions of the selective A_{2A} receptor antagonist, MSX-3 together with a D_1 (SCH23390) or D_2 receptor [S(–) sulpiride] antagonist revealed that catalepsy due to intra-CPu D_1 or D_2 receptor blockade can be potently reversed by an intra-CPu A_{2A} receptor blockade. In conclusion, our results with systemic and intra-CPu drug administration demonstrate that D_1 and D_2 receptor-mediated catalepsy can both be reversed by a concomitant blockade of A_{2A} receptors. Our results implicate that the CPu is a critical neural substrate for antagonistic interactions of a D_1/D_2 receptor blockade and an A_{2A} receptor blockade in control of motor activity. The present results provide further support for the view that A_{2A} receptor antagonists may be potential therapeutics for the treatment of Parkinson's disease.

Introduction

Adenosine is an endogenous neuromodulator acting on four different G-protein-coupled receptor subtypes: A_1, A_{2A}, A_{2B} and A_3 (Fredholm *et al*., 1994). In the basal ganglia, a group of interconnected forebrain nuclei, neuromodulation by adenosine plays a crucial role in motor control (Ferré *et al*., 1997). In the caudate-putamen (CPu), the major input structure of the basal ganglia, A_{2A} receptors are selectively expressed and colocalized with dopamine D_2 receptors in a subpopulation of neurons projecting to the globus pallidus (Schiffmann *et al*., 1991; Fink *et al*., 1992). These striatopallidal neurons constitute the indirect pathway, one of two major output pathways of the CPu controlling the activity of basal ganglia output nuclei, i.e. the substantia nigra pars reticulata and the entopeduncular nucleus. In contrast, striatonigral and striatoentopeduncular neurons regulated by A_1 receptors and D_1 receptors (Ferré *et al*., 1997) constitute the direct basal ganglia pathway. The direct and the indirect pathway have opposing effects on motor activity (Albin *et al*., 1989).

Systemic blockade of A_{2A} receptors stimulated D_1 and D_2 receptor-dependent contralateral rotations in 6-hydroxydopamine lesioned rats (Pinna *et al*., 1996; Pollack & Fink, 1996; Fenu *et al*., 1997). Furthermore, systemic administration of A_{2A} receptor antagonists reversed catalepsy induced by a dopamine receptor blockade or dopamine depletion and potentiated the anticataleptic effects of L-DOPA (Hauber *et al*., 1998; Kanda *et al*., 1994; Kanda *et al*., 1998; Shiozaki *et al*., 1999). The synergistic or antagonistic motor effects mediated by A_{2A} and D_2 receptor ligands could be explained by direct A_{2A}–D_2 receptor interactions on striatopallidal neurons (Ferré *et al*., 1997). In contrast, interactions between the direct and the indirect pathway might account for synergistic or antagonistic motor effects of A_{2A} and D_1 receptor ligands (Pinna *et al*., 1996; Ferré *et al*., 1997) as the respective receptors are located on separate populations of striatal neurons. Recent behavioural data suggest that A_{2A}–D_1 receptor interactions (Popoli *et al*. 2000) might not be as strong as A_{2A}–D_2 receptor interactions (Pinna *et al*., 1996; Pollack & Fink, 1996; Fenu *et al*., 1997; Stromberg *et al*. 2000).

In the present study, we analysed in more detail the effects of an unselective and a selective A_{2A} receptor blockade on catalepsy mediated by a blockade of D_1 or D_2 receptors in rats. The purpose was twofold. First, detailed examination of whether or not an A_{2A} receptor blockade reverses D_1 or D_2 receptor-mediated catalepsy differentially provides deeper insight into the relative strength of the A_{2A}–D_2 and A_{2A}–D_1 interactions in control of motor behaviour. Another purpose was to analyse the role of local A_{2A}–D_2 and A_{2A}–D_1 receptors interactions in the CPu in control of motor behaviour. Most information on motor effects mediated by an A_{2A} receptor blockade relies on studies with systemic drug administration. To characterize the neural substrate of these effects directly, we also performed intra-CPu co-microinfusions of the novel water-soluble A_{2A} receptor antagonist, MSX-3 (Hauber *et al*., 1998; Müller *et al*., 1998) together with a D_1 or D_2 receptor antagonist.

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Materials and methods

Experiments were performed according to the current German Law on Animal Protection and were approved by the proper authorities in Stuttgart, Germany.

Animals

Male Sprague–Dawley rats (Charles-River, Sulzfeld, Germany) weighing 220–250 g on arrival were housed in groups of up to four animals in transparent plastic cages (Type IV; 35 × 55 × 10 cm; Ebeco, Castrop-Rauxel, Germany). In the animal house, temperature (20 ± 2 °C) and humidity (50 ± 10%) were kept constant and a 12 h light : 12 h dark schedule was used with lights on between 06.00 h and 18.00 h. All rats were given *ad libitum* access to water. Standard laboratory maintenance chow (Altromin, Lage, Germany) was restricted to 15 g per animal and day. Experiments were carried out between 09.00 and 16.00 h.

Surgery

For stereotaxic surgery, animals were anaesthetized with sodium pentobarbital (50 mg/kg, i.p.; Sigma-Aldrich, Steelze, Germany) following pretreatment with atropine sulphate (0.05 mg/kg, i.p.; Research Biochemicals Int., Koeln, Germany) and secured in a Kopf stereotaxic apparatus (Kopf Instruments, Tujunga, CA, USA). Bilateral stainless steel cannulae with an outer diameter of 0.8 mm were aimed at the CPu and implanted using standard stereotaxic procedures. The co-ordinates with reference to the atlas of Paxinos & Watson (1986) (toothbar 3.3 mm below the interaural line) were: anteroposterior, 1.7 mm; anterior, bregma; mediolateral, 2.0 mm; dorsoventral, –5.0 mm below the skull. Each rat was given at least 7 days to recover from surgery before catalepsy testing.

Drugs

For systemic administration, SCH23390 [R(+)-7-Chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepin hydrochloride] and theophylline (2,6-dihydroxy-1,3-dimethyl-purine) (Research Biochemicals Int., Koeln, Germany) were dissolved in 0.9% saline, raclopride [S(-)3,5-dichloro-N-(1-ethyl-2-pyrrolidinylmethyl)-2-hydroxy-6-methoxy-benz-amid(+)tartrate] (Astra Arcus, Soedertaelje, Sweden) was dissolved in distilled water. 8-(3-chlorostyryl)caffeine (CSC) (Research Biochemicals Int., Koeln, Germany) was suspended in 0.5% methylcellulose (Fluka, Buchs, Switzerland). Drugs were administered intraperitoneally (i.p.) in a volume of 1 mL/kg. CSC (5 mg/kg) was administered 45 min, SCH23390 (0.75 mg/kg) and raclopride (1.25 mg/kg) 30 min, theophylline (5, 10 mg/kg) 10 min before the onset of behavioural testing; the doses and the timing of injections were chosen to ensure a pronounced drug-induced motor effect (Anderson *et al*., 1995; Hauber & Münkle, 1996; Kafka & Corbett, 1996). The doses of raclopride and SCH23390 were also chosen based on their ability to induce comparable degrees of catalepsy. Administration of respective vehicles in the same volume served as controls.

For bilateral injections into the CPu, MSX-3 (MSX-2 phosphate diNa salt, [3-(3-hydroxypropyl)-8-(3-methoxystyryl)-7-methyl-1-propargylxanthine; Müller *et al*., 1998], SCH23390 (Research Biochemicals Int., Koeln, Germany) and S(–)-sulpiride [(–)-5-(Aminosulphonyl)-*N*-(1-ethyl-2-pyrrodinylmethyl)-2-methoxy-benzamide] (Research Biochemicals Int., Koeln, Germany) were dissolved in 0.9% saline and adjusted to pH of 6.0–7.2. MSX-3 (7 μg) was administered 10 min, SCH23390 (10 μg) and sulpiride (15 μg) 20 min before the onset of behavioural testing in a volume of 1 μL in each hemisphere, respectively. The doses and the timing of injections were chosen to ensure a pronounced drug-induced motor effect (Hauber *et al*., 1998; Hauber & Lutz, 1999). The doses of S(–)-sulpiride and SCH23390 were also chosen based on their ability to induced comparable degrees of catalepsy. For intracerebral injection, S(–)-sulpiride, but not raclopride, was used to block D_2 receptors as S(–)-sulpiride showed only little spread outside the infusion area (Ahlenius *et al*., 1990). For systemic administration, raclopride, but not S(–)-sulpiride, was used to block D_2 receptors, because the cataleptic effects of S(–)-sulpiride had a delayed onset with this route of injection (Elliott *et al*., 1990).

Intracerebral drug infusion

On injection days, the obturators were removed and injection cannulae, with an outer diameter of 0.45 mm, were lowered at the final site of infusion and attached via polyethylene tubing to microliter syringes controlled by a microdrive pump (Kopf Instruments, Tujunga, CA, USA). Drugs and respective vehicles (1.0 μL) were delivered bilaterally at a rate of 0.5 μL/min. Injection cannulae were left in position for an additional 1 min after each infusion to allow for diffusion. Each rat remained in its home cage for an additional 5 min before catalepsy testing.

Catalepsy testing

The standard bar test was used to determine the intensity of catalepsy (e.g. Hoffman & Donovan, 1995). Both forelegs of a rat were placed on a horizontal bar (diameter, 0.7 cm) 9 cm above the surface. The latency from paw placement until the first complete removal of one paw from the support was measured (maximal test duration, 180 s) and termed here as descent latency. If the rat did not assume the position on the bar after three attempts, it received a descent latency of 0 s.

Histology

After completion of behavioural testing, animals were killed by an overdose of sodium pentobarbital (150 mg/kg, i.p.; Sigma-Aldrich, Seelze, Germany) to control for correct placement of cannulae. Brains were rapidly removed, fixed in 10% formalin for 2.5 h and stored in 30% sucrose. Brain sections (20 μm) were cut with a cryostat (Reichert and Jung, Nussloch, Germany), mounted on coated slides and stained with Cresyl Violet. Cannulae placements were verified with reference to the atlas of Paxinos & Watson (1986). Only animals in which cannulae tip placements deviated less than 0.5 mm from target co-ordinates in the CPu were evaluated.

Data analysis

Results are expressed as means ± standard error of the mean (SEM). Data were subjected to a nonparametric statistical analysis using a Mann–Whitney *U*-test (two-tailed) or a Kruskal–Wallis one way analysis of variance (ANOVA) on ranks followed by a multiple comparison procedure after Dunnett's method (equal sample sizes) or Dunn's method (unequal sample sizes). The level of statistical significance (α-level) was set at $P < 0.05$. The SigmaStat (Vers. 2.0, Jandel, Hamburg, Germany) statistical package was used for all statistical computations.

Results

Effects of theophylline and CSC on catalepsy due to systemic D_2 receptor blockade

Systemic blockade of D_2 receptors by raclopride (1.25 mg/kg, i.p.) produced prominent catalepsy which was reversed by systemic coadministration of the unselective A_1/A_2 receptor antagonist

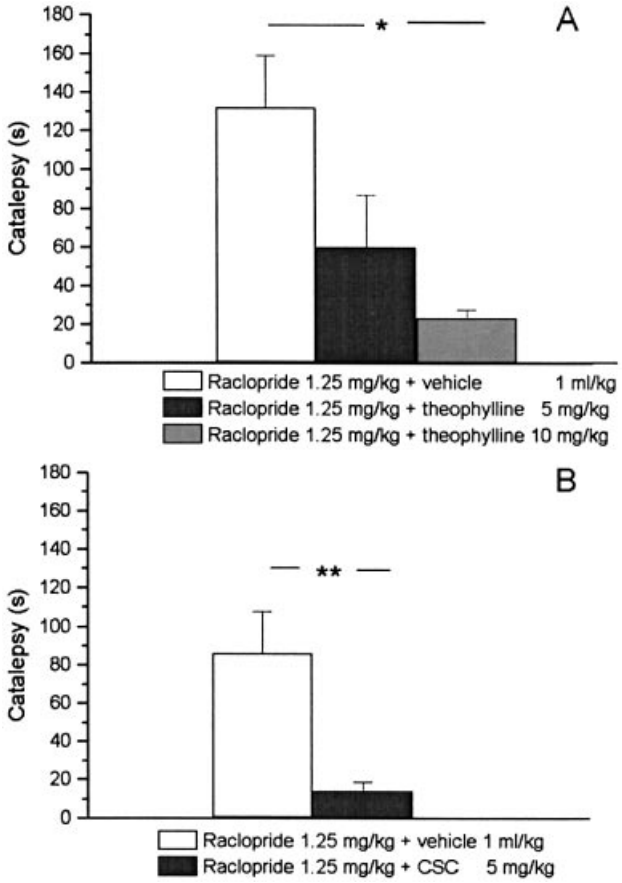

FIG. 1. The effects of theophylline (A) and CSC (B) on raclopride-induced catalepsy determined in the bar test. Intensity of catalepsy was measured as mean descent latency (± SEM). (A) $N = 7$ per group (*$P < 0.05$; ANOVA followed by multiple comparisons after Dunnett's method). (B) $N = 7$–8 per group (**$P < 0.01$; Mann–Whitney U-test).

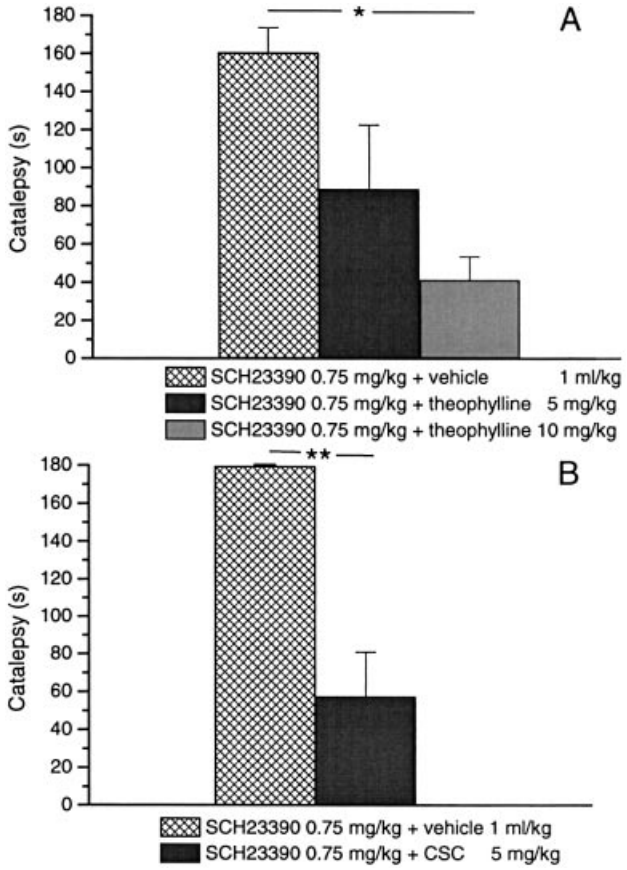

FIG. 2. The effects of theophylline (A) and CSC (B) on SCH23390-induced catalepsy determined in the bar test. Intensity of catalepsy was measured as mean descent latency (± SEM). (A) $N = 5$–7 per group (*$P < 0.05$; ANOVA followed by multiple comparisons after Dunn's method). (B) $N = 8$ per group (**$P < 0.01$; Mann–Whitney U-test).

theophylline. The anticataleptic effect of theophylline was statistically significant after administration of 10 mg/kg (i.p.) (H = 7.78, $P < 0.05$; ANOVA followed by multiple comparisons after Dunnett's method), but not after administration of 5 mg/kg (i.p.) as shown Fig. 1A. Likewise, catalepsy induced by systemic administration of raclopride (1.25 mg/kg, i.p.) was significantly antagonized by systemic coadministration of the selective A_{2A} receptor antagonist, CSC (5 mg/kg, i.p., $P < 0.01$, Mann–Whitney U-test; Fig. 1B).

Effects of theophylline and CSC on catalepsy due to systemic D_1 receptor blockade

Systemic blockade of D_1 receptors by SCH23390 (0.75 mg/kg, i.p.) produced prominent catalepsy which was reversed by systemic coadministration of theophylline (5, 10 mg/kg, i.p.), reaching significance with the higher dose (H = 6.05, $P < 0.05$; ANOVA followed by multiple comparisons after Dunn's method) (Fig. 2A). Furthermore, catalepsy induced by systemic administration of SCH23390 (0.75 mg/kg, i.p.) was antagonized by systemic coadministration of CSC (5 mg/kg, i.p., $P < 0.01$, Mann–Whitney U-test) as depicted in Fig. 2B.

Effects of theophylline and CSC on catalepsy due to intra-CPu D_2 receptor blockade

Intra-CPu blockade of D_2 receptors by S(–)-sulpiride (15 μg in 1 μL per side) produced prominent catalepsy which was reversed by systemic coadministration of the unselective A_1/A_2 receptor antagonist, theophylline. The anticataleptic effect of theophylline was significant after administration of 10 mg/kg (i.p.) (H = 7.68, $P < 0.05$; ANOVA followed by multiple comparisons after Dunn's method), but not after administration of 5 mg/kg (i.p.) as shown Fig. 3A. Likewise, catalepsy induced by intra-CPu infusion of S(–)-sulpiride was significantly antagonized by systemic coadministration of the selective A_{2A} receptor antagonist, CSC (5 mg/kg, i.p., $P < 0.01$, Mann–Whitney U-test; Fig. 3B).

Effects of theophylline and CSC on catalepsy due to intra-CPu D_1 receptor blockade

Intra-CPu blockade of D_1 receptors by SCH23390 (10 μg in 1 μL per side) produced prominent catalepsy which was reversed by systemic coadministration of the unselective A_1/A_2 antagonist, theophylline. The anticataleptic effect of theophylline was significant after administration of 10 mg/kg (i.p.) (H = 8.22, $P < 0.05$; ANOVA followed by multiple comparisons after Dunn's method), but not after administration of 5 mg/kg (i.p.) as shown Fig. 4A. Catalepsy induced by intra-CPu infusion of SCH23390 was not antagonized significantly by systemic coadministration of the selective A_{2A} receptor antagonist CSC (5 mg/kg, i.p., $P > 0.05$, Mann–Whitney U-test; Fig. 4B).

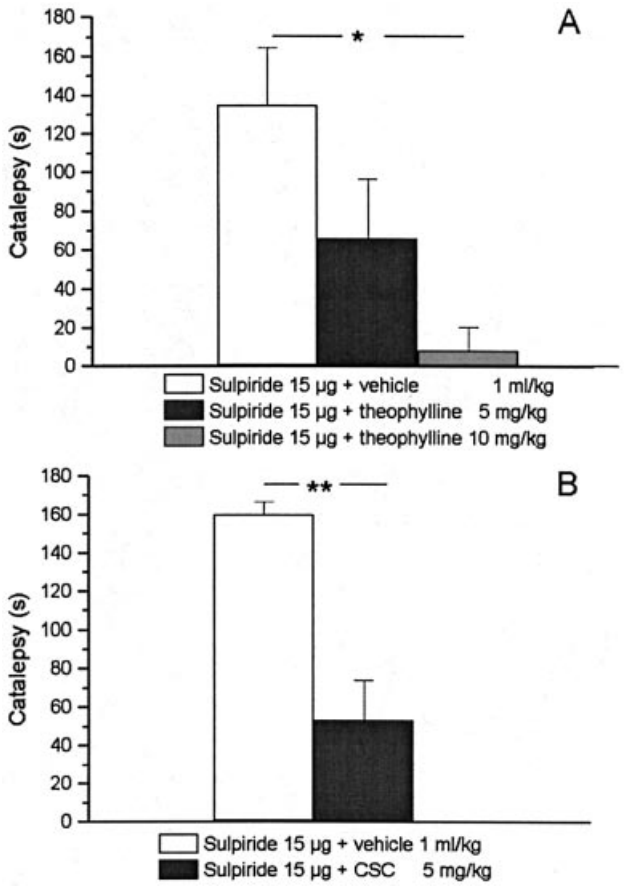

FIG. 3. The effects of theophylline (A) and CSC (B) on catalepsy induced by intra-CPu S(–)-sulpiride determined in the bar test. Intensity of catalepsy was measured as mean descent latency (± SEM). (A) $N = 6–7$ per group (*$P < 0.05$; ANOVA followed by multiple comparisons after Dunn's method). (B) $N = 8–10$ per group (**$P < 0.01$; Mann–Whitney *U*-test).

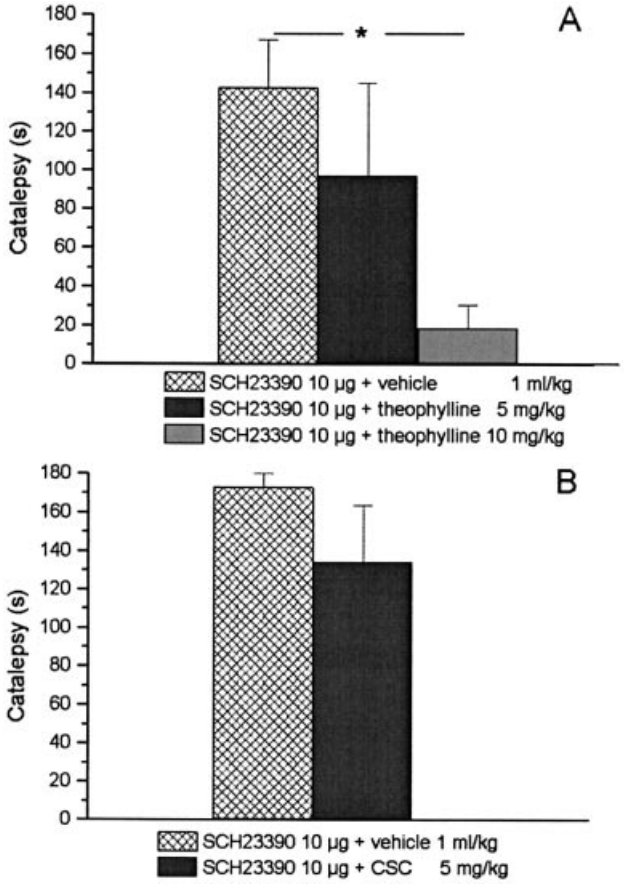

FIG. 4. The effects of theophylline (A) and CSC (B) on catalepsy induced by intra-CPu SCH23390 determined in the bar test. Intensity of catalepsy was measured as mean descent latency (± SEM). (A) $N = 4–8$ per group (*$P < 0.05$; ANOVA followed by multiple comparisons after Dunn's method). (B) $N = 7$ per group.

Effects of MSX-3 on catalepsy due to intra-CPu D_2 and D_1 receptor blockade

As shown in Fig. 5, intra-CPu blockade of D_2 receptors by S(–)-sulpiride (15 µg in 1 µL per side) produced strong catalepsy which was reversed by intra-CPu co-microinfusion of the selective A_2 receptor antagonist MSX-3 ($P < 0.01$, Mann–Whitney *U*-test). In addition, catalepsy after blockade of intra-CPu D_1 receptors by SCH23390 (10 µg in 1 µL per side) was reversed by intra-CPu coinfusion of the selective A_2 receptor antagonist MSX-3 ($P < 0.03$, Mann–Whitney *U*-test).

Histology

In all animals evaluated, cannulae tip placements deviated less than 0.5 mm from target co-ordinates in the CPu. The location of cannulae tips for all rats evaluated are represented in Fig. 6.

Discussion

The main finding of the present study is that catalepsy due to a blockade of D_1 or D_2 receptors within the CPu can be reversed by a concomitant intra-CPu A_{2A} receptor blockade. These behavioural data provide direct evidence for the notion that the CPu is a critical neural substrate for the antagonistic control of motor activity by dopamine and adenosine.

In line with previous data (Casas *et al*., 1988; Kanda *et al*., 1994; Kafka & Corbett, 1996; Mandhane *et al*., 1997), our results demonstrate that catalepsy, induced by a systemic or intra-CPu blockade of D_2 receptors, was potently reversed by systemic administration of the unselective A_1/A_2 receptor antagonist, theophylline and the selective A_{2A} receptor antagonist, CSC. On the other hand, selective A_1 receptor antagonists did not alter catalepsy induced by systemic reserpine or haloperidol (Kanda *et al*., 1994; Mandhane *et al*., 1997). Hence, these findings consistently support the general notion that A_{2A} receptors play a prominent role in anticataleptic responses (Ferré *et al*., 1991; Kanda *et al*., 1994; Hauber *et al*., 1998; Kanda *et al*., 1998; Shiozaki *et al*., 1999).

Our data further reveal that catalepsy, due to systemic or intra-CPu blockade of D_1 receptors, was counteracted by systemic administration of the unselective A_1/A_2 receptor antagonist theophylline. Likewise, the selective A_{2A} receptor antagonist CSC reversed catalepsy induced by a systemic D_1 receptor blockade. These data point to the view that a blockade of A_{2A} receptors might primarily account for the anticataleptic effects of theophylline and CSC in these experiments with a D_1 receptor blockade. However, for unknown reasons, CSC had only minimal effects on catalepsy induced by a blockade of intra-CPu D_1 receptors. This negative result suggests that the particular interaction between CSC and intra-CPu SCH23390 involves yet unidentified mechanisms that might prevent the anticataleptic actions of CSC seen in our other experiments.

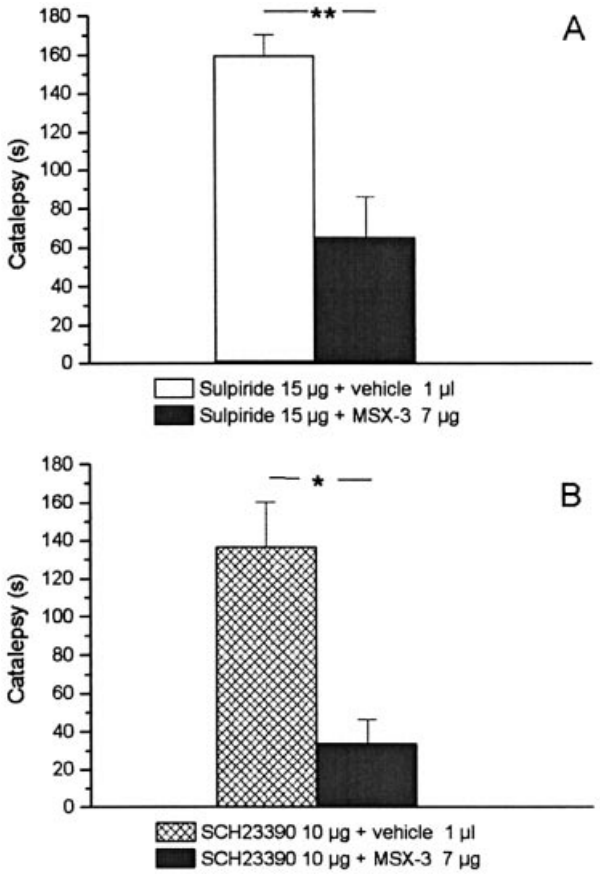

FIG. 5. The effects of intra-CPu co-infusion of MSX-3 on catalepsy induced by intra-CPu S(–)-sulpiride (A) and SCH23390 (B) determined in the bar test. Intensity of catalepsy was measured as mean descent latency (± SEM). (A) $N = 7–8$ per group (**$P < 0.01$; Mann–Whitney U-test). (B) $N = 10$ per group (*$P < 0.05$; Mann–Whitney U-test).

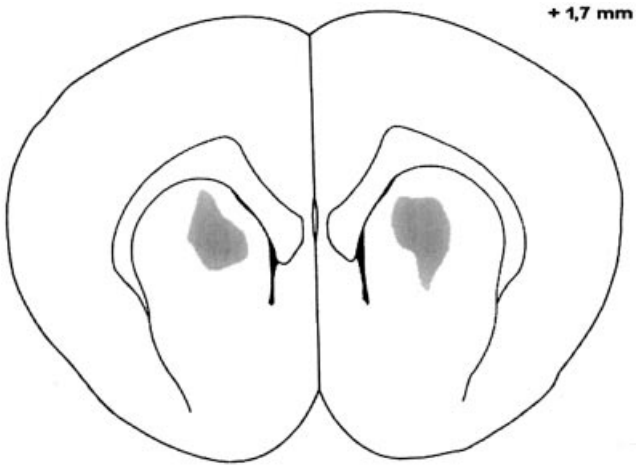

FIG. 6. The shaded areas represent the regions in the CPu where cannulae tips for all rats used for data analysis were located. The plate is an adaptation from the atlas of Paxinos & Watson (1986). The number beside the plate corresponds to millimeters anterior to bregma.

In view of the high densities of D_1, D_2 (Richfield *et al*., 1987) and A_{2A} (Svenningsson *et al*., 1999) receptors in the CPu, it reasonable to assume that this structure is the main neural substrate to mediate the antagonistic motor effects induced by a systemic blockade of D_1 and D_2 receptors vs. A_{2A} receptors. However, this hypothesis is limited because D_1 and D_2 receptors in basal ganglia nuclei downstream of the CPu, i.e. extrastriatal basal ganglia nuclei, play a crucial role in catalepsy as well (Costall *et al*., 1972; Hauber, 1998a; Hauber & Lutz, 1999). Hence, dopamine hypofunction in extrastriatal basal ganglia nuclei also contributes to the expression of catalepsy following systemic manipulations of the dopaminergic system (see Hauber, 1998b). Furthermore, A_{2A} receptors are expressed in moderate to low densities in extrastriatal basal ganglia nuclei (Rosin *et al*., 1998) and interact with D_2 receptors in these structures (Mayfield *et al*., 1993; Mayfield *et al*., 1996; Le Moine *et al*., 1997). Given the extrastriatal A_{2A} receptor expression, a number of possible mechanisms could explain how D_1 and D_2 receptor-dependent catalepsy may be reversed by a concomitant A_{2A} receptor blockade. The first part of this study, as well as other work (e.g. Kanda *et al*. 2000; Kanda *et al*., 1994; Kanda *et al*., 1998) reporting anticataleptic effects of an A_{2A} receptor blockade in animals with dopamine hypofunction, used systemic administration of selective antagonists and, therefore, do not allow direct characterization of the neural substrates mediating the behavioural effects. Thus, in the second part of the present study, we used a novel water-soluble A_{2A} receptor antagonist, MSX-3 (suitable for intracerebral microinfusion) and revealed that blockade of A_{2A} receptors in the CPu potently reversed catalepsy induced by intra-CPu blockade of D_1 as well as D_2 receptors. These findings confirm and extend our previous study (Hauber *et al*., 1998) and provide strong support for the notion that the CPu is a major neural substrate for the antagonistic motor effects mediated by dopamine via D_1/D_2 receptors and adenosine via A_{2A} receptors. However, as noted above, there are additional basal ganglia structures downstream from the CPu which might contribute to the anticataleptic effects of systemically administered A_{2A} receptor antagonists.

The MSX-3-induced reversal of D_2 receptor-mediated catalepsy might be brought about by functionally antagonistic effects of a blockade of A_{2A} and D_2 receptors coexpressed on striatopallidal neurons. These antagonistic effects might involve the well-established intra-membrane interactions of A_{2A} and D_2 receptors (Fuxe *et al*., 1998; Zahniser *et al*. 2000) as well as mechanisms independent of direct receptor–receptor interactions (Richardson *et al*., 1997; Aoyama *et al*. 2000). In addition, it can not be excluded that striatal GABAergic and cholinergic interneurons which express very low levels of A_{2A} receptors (for example, see Richardson *et al*., 1997) contribute to this effect. The intra-CPu blockade of A_{2A} receptors by MSX-3 also reversed D_1 receptor-mediated catalepsy. A_{2A} and D_1 receptors are anatomically separated and functional studies revealed that stimulation of both receptors increased intracellular cAMP in nonoverlapping neuronal populations, presumably striatonigral and striatopallidal neurons (see Svenningsson *et al*., 1999). Because of the segregation of A_{2A} and D_1 receptors, the MSX-3 induced reversal of D_1 receptor-mediated catalepsy might be explained by functionally antagonistic effects of striatopallidal and striatonigral pathways in the output structures. Such network effects within the basal ganglia have been proposed to underlie the A_{2A} receptor antagonist-induced potentiation of D_1 receptor-mediated rotation (Pinna *et al*., 1996; Pollack & Fink, 1996; Fenu *et al*., 1997; also, see Ferré *et al*., 1997).

Our finding that A_{2A} receptor blockade potently reversed catalepsy induced by a D_1 and D_2 receptor blockade is in line with the demonstration that both D_1 and D_2 receptor-mediated rotation was increased by a concomitant A_{2A} receptor blockade in rats (Pinna *et al*., 1996; Pollack & Fink, 1996; Fenu *et al*., 1997). Likewise, A_{2A}

receptor stimulation reversed D_1 as well as D_2 receptor-mediated rotation in rats with unilateral nigrostriatal lesions (Jiang *et al*., 1993; Morelli *et al*., 1994). On the other hand, there is recent evidence that an A_{2A} receptor antagonism did not potentiate D_1 receptor-mediated turning (Popoli *et al*. 2000) as strong as D_2 receptor-mediated turning (Pinna *et al*., 1996; Pollack & Fink, 1996; Fenu *et al*., 1997; Stromberg *et al*. 2000). This difference has been suggested to indicate that interactions between A_{2A} and D_1 receptors at the circuit level are less effective (Stromberg *et al*. 2000) or, alternatively, additional mechanisms between these receptors might account for this difference (Popoli *et al*. 2000). Our results with systemic and intra-CPu drug administration provide no evidence that D_1 and D_2 receptor-mediated catalepsy was differentially reversed by an A_{2A} receptor blockade. However, the data do not allow us to conclude that an A_{2A} receptor blockade reversed D_1 and D_2 receptor-mediated catalepsy to the same extent, because the degrees of D_1 and D_2 receptor-mediated catalepsy were variable throughout our experiments and only a limited dose–response range of A_{2A} and dopamine receptor antagonists was tested. Nevertheless, the data imply that striatal A_{2A} receptors are tonically activated and that their blockade produced changes in the activity of striatopallidal neurons strong enough to compensate for both D_1 and D_2 receptor-mediated hypokinesia. The underlying antagonistic mechanisms are probably different in that they are located either at a common neuronal substrate (A_{2A}–D_2 receptors) or at the network level (A_{2A}–D_1 receptors).

In summary, using intracerebral co-microinfusions the present study provides, to the best of our knowledge, the first direct evidence that the CPu is a critical neural substrate which mediates the antagonistic motor effects of an A_{2A} receptor blockade and a D_1 or D_2 receptor blockade. The data add further support to the notion that A_{2A} receptor antagonists may be useful therapeutics for the treatment of Parkinson's disease (Richardson *et al*., 1997; Mally & Stone, 1998).

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Abbreviations

CPu, (Caudate-Putamen); CSC, 8-(3-chlorostyryl) caffeine; MSX-3, (3-hydroxypropyl)-8-(3-methoxystyryl)-7-methyl-1-propargylxanthine; SCH23390, [R(+)-7-Chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepin hydrochloride].

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