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# Motor depressant effects mediated by dopamine $D_2$ and adenosine $A_{2A}$ receptors in the nucleus accumbens and the caudate-putamen

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**Abstract**

We compared hypolocomotion and catalepsy mediated by striatal dopamine $D_2$ and adenosine $A_{2A}$ receptors using microinfusions of the adenosine $A_{2A}$ receptor agonist 2-*p*-(2-carboxyethyl) phenethylamino-5′-*N*-ethylcarboxamidoadenosine hydrochloride (CGS21680) and the dopamine $D_2$ receptor antagonist raclopride into the nucleus accumbens and the caudate-putamen. The effective doses ($ED_{25/50}$) of CGS21680 and raclopride which produced equivalent reductions of spontaneous locomotion after microinfusion into the nucleus accumbens were found to induce similar degrees of catalepsy after microinfusion into the caudate-putamen. This comparable, little separation of the effective doses of a dopamine $D_2$ receptor antagonist and an adenosine $A_{2A}$ receptor agonist to produce locomotor inhibition and catalepsy support the idea that adenosine $A_{2A}$ receptor agonists as potential antipsychotic agents may have a similiar therapeutic profile as dopamine $D_2$ receptor antagonists. 

*Keywords:* Dopamine $D_2$ receptor; Adenosine $A_{2A}$ receptor; Striatum; Locomotion; Catalepsy; (Rat)

## 1. Introduction

Adenosine has been shown to modulate central neurotransmission through actions on distinct classes of cell-surface receptors, which are divided into two major subtypes, $A_1$ and $A_2$. Adenosine $A_2$ receptors are further subdivided into $A_{2A}$ and $A_{2B}$ subtypes (Collis and Hourani, 1993). Adenosine $A_{2A}$ receptors are abundantly expressed in the nucleus accumbens and in the caudate-putamen (Jarvis and Williams, 1989; Schiffmann et al., 1991) and modulate striatal dopaminergic neurotransmission. Important mechanisms underlying the effects of adenosine on dopaminergic neurotransmission are antagonistic adenosine $A_{2A}$-dopamine $D_2$ receptor interactions: activation of adenosine $A_{2A}$ receptors decreases both the affinity of dopamine $D_2$ receptors and the signal transduction from dopamine $D_2$ receptors to the guanine nucleotide-binding protein (for review, see Fuxe et al., 1995). Antagonistic adenosine-dopamine interactions in the striatum may account for motor depressant effects induced by adenosine $A_{2A}$ receptor agonists and their reversal by dopamine $D_2$ receptor agonists (Ferré et al., 1991; Morelli et al., 1994) and may also explain results from microdialysis experiments showing that co-infusion of an adenosine $A_{2A}$ receptor agonist potently counteracted the reduced pallidal γ-aminobutyric acid levels following striatal infusion of a dopamine $D_2$ receptor agonist (Ferré et al., 1993).

Using quantitative receptor autoradiography, a recent study suggests that more powerful antagonistic adenosine $A_{2A}$-dopamine $D_2$ receptor interactions might exist in the ventral striatum as compared with the dorsal striatum (Ferré et al., 1994). Accordingly, infusion of an adenosine $A_{2A}$ receptor agonist into the nucleus accumbens might have more pronounced effects on dopamine $D_2$ receptor-mediated motor behaviour than infusion into the caudate-putamen. It is well known that dopamine $D_2$ receptors within the nucleus accumbens are involved in locomotor control (Van den Boss et al., 1988), while dopamine $D_2$ receptors in the caudate-putamen mediate catalepsy (Calderon et al., 1988). We examined locomotor and cataleptogenic effects of the selective adenosine $A_{2A}$ receptor agonist CGS21680 after microinfusion into the nucleus accumbens and caudate-putamen in comparison to the selective dopamine $D_2$ receptor antagonist raclopride. If adenosine-dopamine interactions are more powerful in the nucleus accumbens, then, local infusion of a dose of

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CGS21680 which significantly reduces locomotion should be largely devoid of cataleptogenic actions.

## 2. Materials and methods

### 2.1. Subjects

Male Sprague-Dawley rats (Interfauna, Tuttlingen, Germany) weighing 240–260 g on arrival were used. They were housed in groups of four to five animals per cage and maintained on a 12:12 h light/dark cycle (lights on at 06.00–18.00 h) with a room temperature of $22 \pm 3$°C. Each animal received 15 g of food per day (maintenance diet; Altromin, Lage, Germany) and had free access to water.

### 2.2. Stereotaxic surgery

For stereotaxic surgery, animals were anaesthetized with sodium pentobarbital (50 mg/kg, i.p.) following pretreatment with atropine sulphate (0.05 mg/kg, i.p.) and placed in a Kopf stereotaxic frame. Bilateral stainless steel cannulae (outer diameter 0.8 mm) were aimed at the dorsal or ventral striatum and implanted using standard stereotaxic procedures. The co-ordinates for the caudate-putamen are: anterioposterior: 1.7 mm anterior to bregma, mediolateral: 2 mm; dorsoventral: 5.5 mm above the interaural line with the toothbar 3.3 mm below interaural line (Paxinos and Watson, 1986). The coordinates for the nucleus accumbens are: anterioposterior: 3.8 mm anterior to bregma, mediolateral: 1.7 mm, dorsoventral −6.5 mm below the skull surface with the toothbar 5 mm above the interaural line (Pellegrino et al., 1981).

### 2.3. Microinfusion of drugs

Behavioural testing was started after a recovery period of 3–4 days. Bilateral injection cannulae (outer diameter 0.45 mm) were lowered at the final site of infusion and attached via polyethylene tubing to microlitre syringes controlled by a microdrive pump (Kopf Instruments, Tujunga, CA, USA). The infusions were made bilaterally in a volume of 1 μl and delivered over 1 min. A period of 1 min was allowed for diffusion before the injection cannulae were removed. The selective adenosine $A_{2A}$ receptor agonist 2-*p*-(2-carboxyethyl) phenethylamino-5′-*N*-ethylcarboxamidoadenosine hydrochloride (CGS21680; Ciba-Geigy, Summit, NJ, USA) and the dopamine $D_2$ receptor antagonist raclopride (Astra Arcus, Södertälje, Sweden) were diluted in distilled water and administered in a volume of 1 μl. Infusions were made 60 min (CGS21680) or 20 min (raclopride) before the onset of behavioural testing. The timing of the injections was chosen according to the respective peak motor effects of both compounds. While raclopride had a rapid onset of action lasting for about 30 min (see also Van den Boss et al., 1988), CGS21680 had a slower onset of action detectable 15 min after microinfusion for at least 120 min with a peak about 60 min after microinfusion (Hauber, unpublished results; see also Ferré et al., 1991; Vellucci et al., 1993).

### 2.4. Behavioural testing

Catalepsy was assessed using two tests: (i) bar: both forelegs were placed on a horizontal bar (9 cm above the surface); (ii) grid: an animal was placed on a vertical wire grid. The latency from paw placement until the first complete removal of one paw from the support was measured (maximum 180 s) and termed here as descent latency. Locomotor activity was measured in an open field ($69 \times 69$

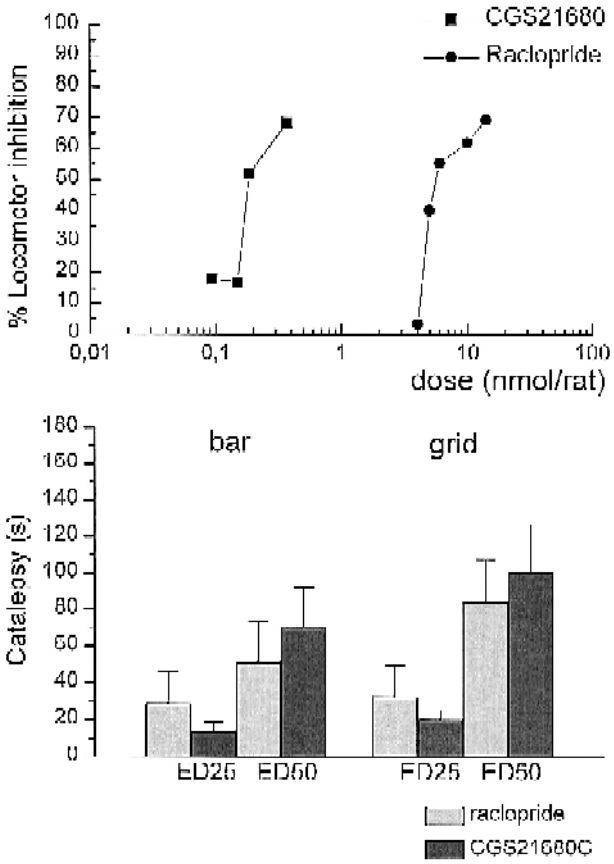


Fig. 1. Motor effects of intracerebral infusions of the dopamine $D_2$ receptor antagonist raclopride and of the adenosine $A_{2A}$ receptor agonist CGS21680C. A: Bilateral infusions of raclopride and CGS21680 into the nucleus accumbens dose-dependently inhibited locomotor activity in an open field. The $ED_{25/50}$ values for CGS21680 were 0.125/0.231 nmol/rat, for raclopride 4.54/6.9 nmol/rat, respectively. $n = 6–7$ per group. B: Bilateral infusions of the $ED_{25/50}$ of raclopride and CGS2180C into the caudate-putamen produced a similar degree of catalepsy in the bar and in the grid test as measured by the mean descent latencies (s) (±S.E.M.). Mean descent latencies of both groups differed insignificantly (Mann-Whitney *U*-test). $n = 10$ per group.

cm) divided by lines into nine squares. The number of line crossings during a 5-min session was counted.

### 2.5. Histology

For histology, an overdose of sodium pentobarbital (150 mg/kg, i.p.) was used to kill the animals. Brains were removed, fixed in 10% formalin for 2.5 h and stored in 30% sucrose. Brain sections (20 μm) were cut with a cryostat (Reichert & Jung, Stuttgart, Germany), mounted on coated slides and stained with Cresyl Violet to control for cannulae placements. Only animals in which cannula tip placements deviated less than 0.5 mm from target coordinates were evaluated.

### 2.6. Data evaluation and statistics

Data are expressed as means (± standard errors of the means, S.E.M.). Catalepsy data were analysed using the Mann-Whitney *U*-test (two-tailed). Activity counts from the open field were subjected to a one-way analysis of variance (ANOVA). $P < 0.05$ was considered to represent significant differences. Effective doses for locomotor inhibition by 25 and 50% ($ED_{25/50}$) were calculated by non-linear regression analysis (GB-STAT, Version 5.0, Dynamic Microsystems, USA).

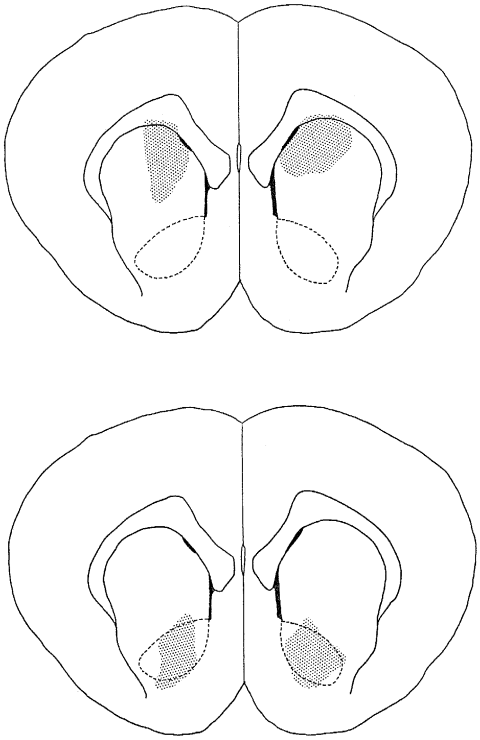

Fig. 2. Schematic representation of the range of cannula tip placements in the caudate-putamen and in the nucleus accumbens from all animals evaluated.

## 3. Results

Local administration of CGS21680 or raclopride into the nucleus accumbens reduced dose-dependently spontaneous locomotion (Fig. 1, Table 1). On the basis of these data, the effective doses for both drugs to reduce locomotion by 25 or 50% were calculated. The $ED_{25/50}$ values for CGS21680 were 0.13/0.23 nmol/rat, for raclopride 4.54/6.90 nmol/rat, respectively. The $ED_{25/50}$ for CGS21680 and raclopride produced a similar degree of catalepsy in the grid and bar test after infusion into the caudate-putamen (Fig. 1). The precision of microinfusions was verified by localization of cannula tip placements as shown in Fig. 2.

Table 1
Effects of intra-accumbal infusions of raclopride or CGS21680 on spontaneous locomotor activity measured during a 5-min session in an open field

| Drug | Line crossings | Drug | Dose (nmol) | Line crossings | Significance |
|---|---|---|---|---|---|
| Saline | 25.2 ± 5.5 | Raclopride | 4 | 24.8 ± 8.0 | |
| Saline | 16.7 ± 2.0 | Raclopride | 5 | 10.0 ± 3.1 | |
| Saline | 65.8 ± 11.3 | Raclopride | 6 | 29.3 ± 13.7 | |
| Saline | 48.5 ± 9.9 | Raclopride | 11 | 18.4 ± 5.5 | 0.01 |
| Saline | 47.3 ± 6.3 | Raclopride | 15 | 14.5 ± 3.4 | 0.001 |
| Saline | 16.8 ± 4.5 | CGS21680 | 0.09 | 13.8 ± 5.1 | |
| Saline | 13.0 ± 4.2 | CGS21680 | 0.15 | 10.8 ± 7.6 | |
| Saline | 33.0 ± 8.1 | CGS21680 | 0.19 | 15.8 ± 4.5 | |
| Saline | 39.0 ± 9.0 | CGS21680 | 0.37 | 12.3 ± 5.0 | 0.02 |

Drug doses refer to the dose per animal. Microinfusions were made in a volume of 1 μl per side. Line crossings (means ± S.E.M.) were compared using a one-way analysis of variance (ANOVA). $n = 6–7$ per group.

## 4. Discussion

The present results demonstrate that blockade of dopamine $D_2$ receptors or stimulation of adenosine $A_{2A}$ receptors in the nucleus accumbens both reduce spontaneous locomotion. CGS21680 had an $ED_{50}$ of 0.23 nmol/rat for locomotor inhibition which is markedly higher than those for mice which is about 0.002 nmol(Barraco et al., 1994). This difference most probably reflects species differences. In rats substantially higher doses (about more than 4 nmol per animal) are needed to induce massive motor effects through intrastriatal (Hauber and Münkle, 1995; Vellucci et al., 1993) or intracerebroventricular infusion (Ferré et al., 1991) of CGS21680. The $ED_{50}$ of 6.9 nmol/rat determined for raclopride is comparable to the dose range found to decrease spontaneous locomotor activity in rats after intra-accumbal infusion in other studies (Van den Boss et al., 1988). Thus, adenosine $A_{2A}$ and dopamine $D_2$ receptors seem to be important components of the ventral striatal motor system (Ferré et al., 1994).

Furthermore, raclopride and CGS21680 were found to induce catalepsy if administered locally into the caudate-putamen. These findings extend previous studies showing the involvement of dopamine $D_2$ receptors (Calderon et al., 1988; Ossowska et al., 1990) and adenosine $A_{2A}$ receptors (Hauber and Münkle, 1995) of the caudate-putamen in mediating cataleptic responses. A contribution of drug diffusion to the motor effects measured here seems unlikely, because hypolocomotion was selectively produced by dopamine depletion in the nucleus accumbens, but not in the caudate-putamen (Kelly et al., 1975), while akinetic effects were observed only after dopamine depletion in the caudate-putamen, but not in the nucleus accumbens (Amalric and Koob, 1987).

The most significant finding of the present study is that doses of CGS21680 or raclopride which, after infusion into the nucleus accumbens, produce equivalent reductions of spontaneous locomotion, induce similar degrees of catalepsy after local infusion into the caudate-putamen. A major mechanism which might account for the CGS21680-induced motor inhibition are antagonistic interactions between adenosine $A_{2A}$ receptors and dopamine $D_2$ receptors described in the ventral and dorsal striatum (Ferré et al., 1993, 1994). From recent studies in striatal membrane preparations it was inferred that adenosine $A_{2A}$-dopamine $D_2$ receptor interactions might be stronger in the ventral striatum than in the dorsal striatum (Ferré et al., 1994). The implication is that dopamine $D_2$ receptor-dependent motor behaviour might be suppressed by a given dose of an adenosine $A_{2A}$ receptor agonist more effectively when administered to the ventral striatum as compared to the dorsal striatum. However, the present results show that CGS21680 exerted similar effects as raclopride on dopamine $D_2$ receptor-dependent motor behaviour mediated by nucleus accumbens and caudate-putamen. CGS21680 appears to have no preferential action in the ventral striatum, because doses which substantially inhibit locomotion are associated with pronounced cataleptic effects. Thus, these data do not point to a different strength of adenosine $A_{2A}$-dopamine $D_2$ receptor interactions in the nucleus accumbens and the caudate-putamen. On the other hand, the effects of CGS21680 may not exclusively be mediated by a modulation of dopamine $D_2$ receptor-mediated transmission. Although it is assumed that the regulation of signaling via dopamine $D_2$ receptors is a major effect of adenosine $A_{2A}$ receptor stimulation, adenosine may alter the activity of striatal neurons independent of its ability to modulate dopamine $D_2$ receptor-mediated transmission (Svenningsson et al., 1995). Therefore, a dopamine-independent mechanism may also contribute to the observed CGS21680-induced motor inhibition. However, its relative importance and possible differences within the dorsal and ventral striatum are not yet determined.

Taken together, the present data confirm that adenosine $A_{2A}$ and dopamine $D_2$ receptors in the nucleus accumbens and the caudate-putamen both mediate motor inhibition, but reveal that the respective doses of an adenosine $A_{2A}$ receptor agonist and a dopamine $D_2$ receptor antagonist necessary to produce hypolocomotion and catalepsy show a comparable, little separation. In line with this conclusion, CGS21680 was found to have a behavioural profile in the apomorphine-induced climbing mouse assay and the catalepsy test similar to raclopride and other dopamine receptor antagonists (Kafka and Corbett, 1996). In this study with systemic drug administration, CGS21680 antagonized apomorphine-induced climbing behaviour and caused catalepsy in a similar fashion as raclopride or haloperidol with little separation of the effective doses required to produce these two effects.

A blockade of dopamine $D_2$ receptor in nucleus accumbens and other parts of the limbic system may be associated with the antipsychotic activity of neuroleptics, while the blockade of dopamine $D_2$ receptors in the caudate-putamen may account for their extrapyramidal motor side effects (see Jackson et al., 1994 for a recent review). In view of the more powerful antagonistic adenosine $A_{2A}$-dopamine $D_2$ receptor interactions in the nucleus accumbens as compared to the caudate-putamen (Ferré et al., 1994), adenosine $A_{2A}$ receptor agonists may be potential antipsychotics with a low incidence of extrapyramidal side effects. The present finding of a comparable, little separation of the effective doses of a dopamine $D_2$ receptor antagonist and an adenosine $A_{2A}$ receptor agonist to produce locomotor inhibition and catalepsy after striatal microinfusion supports the idea that adenosine $A_{2A}$ receptor agonists as potential antipsychotic agents may have a similiar therapeutic profile as dopamine $D_2$ receptor antagonists.

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