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The effects of dopamine D3 agonists and antagonists in a nonhuman primate model of tardive dyskinesia

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Abstract

Tardive dyskinesia (TD), a serious complication of antipsychotic dopamine (DA) antagonist treatment, has been hypothesised to develop due to a dominant DA D1 relative to DA D2 receptor function. Recent genetic and pharmacological studies implicate the DA D3 receptor in TD. The present study examined the role of the DA D3 receptor in relation to the DA D1/D2 imbalance hypothesis of TD in nonhuman primates. Eight *Cebus* monkeys displaying mild to severe TD due to previous chronic exposure to DA D2 antagonists were acutely injected with SKF 81297 (DA D1 agonist) 0.3 and 0.6 mg/kg, pramipexole (DA D3>D2 agonist) 0.025–0.1 mg/kg, CIS-8-OH-PBZI (DA D3 agonist) 5–10 mg/kg and SB-27701-A (DA D3 antagonist) 1–5 mg/kg and rated for oral dyskinesia. SKF 81297, 0.3 and 0.6 mg/kg, exacerbated TD. Pramipexole and CIS-8-OH-PBZI reduced SKF 81297-induced TD, while SB-27701-A had no effect. When administered alone, SB-27701-A increased TD relative to placebo, while pramipexole and CIS-8-OH-PBZI had no significant effect. Pramipexole did, however, ameliorate TD in those monkeys with severe TD. These results point towards a role of the DA D3 receptor in TD, but indicate that the DA D2 receptor may also play an essential role.

Keywords: Dopamine D1 agonist; Dopamine D3 agonist; Dopamine D3 antagonist; Tardive dyskinesia; Monkey

1. Introduction

Tardive dyskinesia (TD) is a common complication of long-term neuroleptic use and a particularly serious side effect due to its potential irreversibility. The main theories explaining its development include dopamine (DA) receptor hypersensitivity (Klawans et al., 1980), GABA insufficiency (Fibiger and Lloyd, 1984) and a neurotoxic effect of chronic antipsychotic medication (Gerlach, 1977), e.g., through production of free radicals (Cadet and Lohr, 1989). Another theory suggests that TD may be due to an imbalance of DA D1 receptor function in relation to DA D2 receptor function (Rosengarten et al., 1983; Gerlach and Casey, 1988; Peacock et al., 1990; Peacock and Gerlach, 1997).

The isolation of DA D2 (DA D2 long and short, D3 and D4) and D1 receptor subtypes (D1 and D5; Sibley et al., 1993) and the development of subtype specific ligands allow further investigation of dopaminergic theories of dyskinesia.

The present study focuses upon the role of the DA D3 receptor in the pathogenesis of TD. This receptor has received increasing attention as a potential target for antiparkinsonian and antipsychotic drugs (Joyce, 2001). Thus, pramipexole, an antiparkinsonian agent, has been found to predominantly stimulate DA D3 vs. DA D2 receptors (Mierau et al., 1995). DA D3 alleles have been implicated in sensitivity to TD (Steen et al., 1997) and akathisia (Eichhammer et al., 2000) and DA agonists with preference for DA D3 receptors relative to DA D2 receptors (PD 128,907; CIS-8-OH-PBZI) have shown antipsychotic potential (Witkin et al., 1998; Fink-Jensen et al., 1998).

We hypothesised that DA D3 agonism would ameliorate dyskinesia and that DA D3 antagonism would either have no effect or result in an exacerbation. To test these hypotheses, we used eight *Cebus* monkeys, all displaying mild to severe oral dyskinesia. The monkeys were acutely treated with the mixed DA D3/D2 agonist pramipexole, the specific DA D3 agonist CIS-8-OH-PBZI and the specific DA D3 antagonist SB-277011-A, given alone and in combination with the DA D1 agonist SKF 81297, a substance previously shown to

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augment oral dyskinesia in *Cebus* monkeys (Lublin and Gerlach, 1988; Lublin, 1995).

2. Methods

2.1. Animals

Six male and two female *Cebus apella* monkeys, weighing 2.25–4.95 kg, were used. The monkeys were pooled from two groups, one which had been primed through prior chronic treatment with haloperidol (Peacock and Gerlach, 1999) and the other through prior chronic treatment with raclopride (Gerlach and Hansen, 1993). Three monkeys had mild, two had moderate, and three had severe TD. The animals had been free of medication for 3 months prior to the present investigation. During the study, the monkeys were housed in separate cages (L × W × H: 0.83 × 0.76 × 1.30 m) under a 12-h light/dark cycle, in a temperature- and humidity-regulated environment. Visual, olfactory and auditory contact between the monkeys was possible during and between experiments. The animals were afforded full veterinary care, both by an on-site and an off-site veterinarian in case of medical emergencies. An external veterinarian ensured that the study complied with the European Communities Council Directive of Nov. 24th, 1986 (86/609/EEC) and with the Danish law regulating experiments on animals.

2.2. Drugs and design

The drugs used were the selective D1 agonist SKF 81297 (2,3,4,5-tetrahydro-6-chloro-1-phenyl-1H-3-benzazepine-7,8-diol), the selective D3 agonist CIS-8-OH-PBZI (Scheideler et al., 1997), the mixed D2/D3 agonist with relative D3 receptor preference over D2 receptor preference pramipexole (=SND 919; 2-amino-4,5,6,7-tetrahydro-6-propyl-amino-benzthiazole-dihydrochloride; Mierau et al., 1995), the selective D3 antagonist SB-277011-A {*trans*-*N*-[4-[2-(6-cyano-1,2,3,4-tetrahydroisoqunolin-2-yl)ethyl]cyclohexyl]-4-quinolininecarboxamide}(Reavill et al., 2000; Austin et al., 2001) and saline as placebo.

Table 1
Description of behaviors and rating scales

Behavior	Description	Scale
Unrest	Restlessness including fidgeting and frequent changes of direction of movement or frequent changes between different behaviors.	0–6
Stereotypies	Persistently repetitive senseless activity, lacking variation.	0–6
Arousal	Degree of vigilance ranging from not awake to extreme vigilance in relation to self or the environment.	0–6
Locomotion	Movement in space.	0–6
Sedation	Degree of drowsiness ranging from fully awake to heavy sleeping (cannot be awakened by gross stimuli).	0–6
Oral dyskinesia	Jaw movements and tongue protrusions.	Counts/90 s
Bradykinesia	Slow and/or stiffened movements ranging from normal tempo and flexibility to fixed maintained postures.	0–6
Dystonia	Clonic movement of head, neck, limbs and trunk. Gaping and grimacing.	0–6

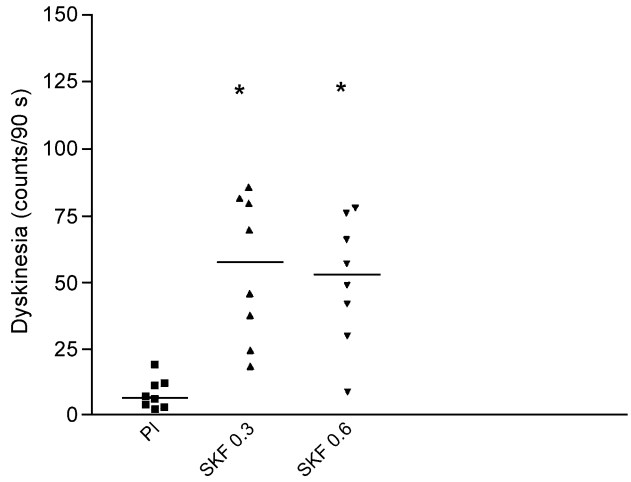

Fig. 1. Oral dyskinesia expressed in counts/90 s after treatment with saline as placebo (Pl) and with SKF 81297 (SKF). The bar indicates the median score for the group over 60 min; the symbols represent the score for each monkey. The doses in milligrams per kilogram are indicated below the columns. * $P < .05$ as compared to placebo.

The drugs were dissolved in saline immediately before their subcutaneous injection and given both alone or combined (simultaneously by separate injection) with a fixed dose of SKF 812970 (0.3 mg/kg), as that dose of SKF 81297 has previously been shown to result in a significant exacerbation of TD (Lublin, 1995). To test for a potential "ceiling" effect of SKF 81297 (Peacock et al., 1990), 0.6 mg/kg was also given alone. CIS-8-OH-PBZI was given at 5.0, 7.5 and 10.0 mg/kg (due to limited access to the substance, only 10 mg/kg being tested alone), the doses determined in pilot trials of the drug's effects in the animals. The doses of pramipexole used were 0.025, 0.05 and 0.1 mg/kg (Peacock and Gerlach, 1993). SB-277011-A was given at 1.0, 2.0, 4.0 and 5.0 mg/kg, based upon estimates in accord with experience as to the animal's sensitivity as determined in earlier experiments in our laboratory (Peacock and Gerlach, 1999).

Placebo and SKF 81297 0.3 mg/kg, alone, were each given twice, at the beginning and at the end of the study. The monkeys were tested at an interval of 2–3 days, at the same time each day.

2.3. Evaluation

The behavior of the monkeys was video recorded in 90-s sequences, at Time 0 (before injection) and at 15-min intervals for a period of 60 min (after injection). Oral dyskinesia was scored by actual counts per 90-s time period

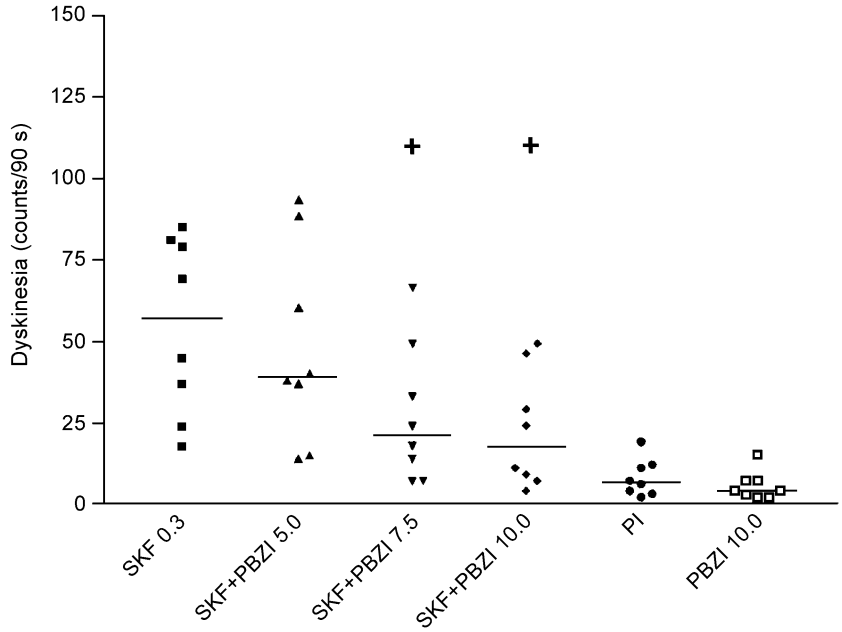

Fig. 2. Oral dyskinesia expressed in counts/90 s after treatment with saline as placebo (Pl), SKF 81297 (SKF) and CIS-8-OH-PBZI (PBZI). The bar indicates the median score for the group over 60 min; the symbols represent the score for each monkey. The doses in milligrams per kilogram are indicated below the columns. $^{+}P<.05$ as compared to SKF 81297 0.3 mg/kg.

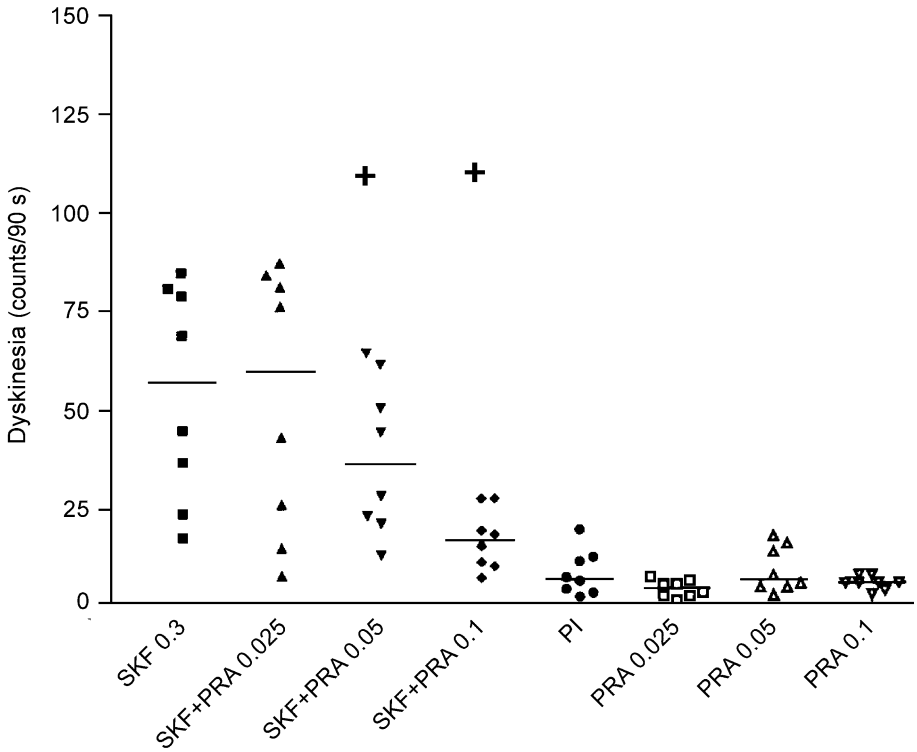

Fig. 3. Oral dyskinesia expressed in counts/90 s after treatment with saline as placebo (Pl), SKF 81297 (SKF) and pramipexole (PRA). The bar indicates the median score for the group over 60 min; the symbols represent the score for each monkey. The doses in milligrams per kilogram are indicated below the columns. $^{+}P<.05$ as compared to SKF 81297 0.3 mg/kg.

while other behaviors were rated on a six-point scale (Table 1; Andersen et al., 2002, 2003; Peacock et al., 1990; Peacock and Gerlach, 1993). All parameters were rated by two observers who were blind to the treatment of the animals.

3. Statistical analysis

The effect of the drugs is indicated by scatter plots, expressing the median of the scores for the eight animals over the first 60 min after drug injection with a bar, and where each individual animal's score is represented by a symbol (Brotchie and Fox, 1999). The results were analysed for overall treatment effects by means of the nonparametric Friedman's test for repeated measures followed by Wilcoxon's paired test for nonparametric data, when Friedman's test was significant. The accepted level of significance was $P < .05$ for all tests.

4. Results

During placebo (Fig. 1), all eight animals displayed mild to severe oral dyskinesia, consisting of tongue protrusions, licking and smacking movements and grinding of the jaws. Placebo produced no significant difference in the character or degree of oral dyskinesia as compared to baseline. There was no significant difference in oral dyskinesia during the placebo trials at the beginning and end of the study.

SKF 81297 (0.3 mg/kg) exacerbated oral dyskinesia compared to placebo, SKF 81297 (0.6 mg/kg) resulting in no further increase of oral dyskinesia compared to the lower dose (Fig. 1). There was no significant difference in the effect of SKF 81297 0.03 mg/kg, given at the beginning and end of the trial.

Fig. 2 shows that CIS-8-OH-PBZI 7.5 and 10 mg/kg reduced SKF 81297-induced oral dyskinesia, while 5 mg/kg had no significant effect. CIS-8-OH-PBZI, 10.0 mg/kg alone, had no effect on oral dyskinesia compared to placebo (Fig. 2). Pramipexole, 0.05 and 0.1 mg/kg, also reduced oral movements induced by SKF 81297 (Fig. 3), while 0.025 mg/kg had no significant effect. Pramipexole, administered alone, showed no difference compared to placebo at any dose. In Fig. 4, it can be seen that SB-277011-A, administered alone, aggravated oral dyskinesia compared to placebo at 2, 4 and 5 mg/kg, while 1 mg/kg had no significant effect. SB-277011-A did not significantly affect SKF 81297-induced dyskinesia at any dose.

None of the compounds affected unrest, stereotypies, arousal, locomotion, sedation, bradykinesia or dystonia at any dose compared to placebo.

CIS-8-OH-PBZI, administered alone, induced emesis in five monkeys at 10.0 mg/kg. When combined with SKF 81297, emesis was only observed in one monkey.

5. Discussion

As stated in the introduction, the DA D3 receptor has gained increased focus as a possible mediator of movement

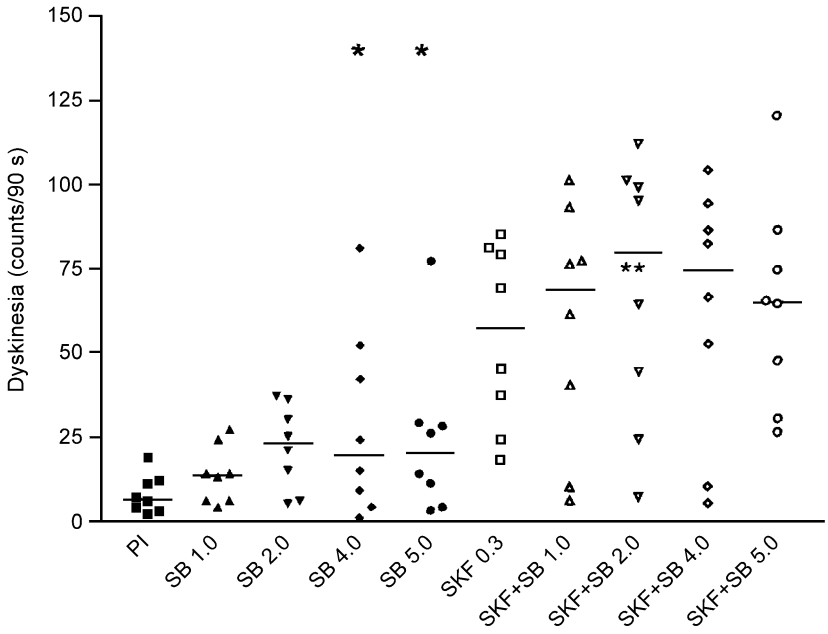

Fig. 4. Oral dyskinesia expressed in counts/90 s after treatment with saline as placebo (Pl), SKF 81297 (SKF) and SB-277011-A (SB). The bar indicates the median score for the group over 60 min; the symbols represent the score for each monkey. The doses in milligrams per kilogram are indicated below the columns. * $P < .05$ as compared to placebo.

disorders, i.e., dyskinesia, akathisia and parkinsonism. In earlier studies in our laboratory (Peacock et al., 1990; Lublin and Gerlach, 1988), we have found evidence concurring with the proposal of Rosengarten et al. (1983) that TD might be the result of an imbalance of dopaminergic receptor function, due to a dominant DA D1 relative to DA D2 receptor function. As selective DA D3 ligands have now become available for research, we were interested in investigating the potential role of the DA D3 receptor in relation to the DA imbalance hypothesis.

In the present study, we did indeed find an antidyskinetic effect of the two DA D3 agonists in relation to DA D1 agonist induced dyskinesia. The inhibition of dyskinesia occurred at doses of each of the DA D3 agonists that had no other observable behavioral effects compared to placebo. While earlier studies in this laboratory (Peacock et al., 1990; Lublin and Gerlach, 1988; Peacock and Gerlach, 1993) could not rule out that TD suppression was due to a behavioral-activating effect of DA D2 agonists (e.g. quinpirole or SND 919), the present study points to a specific inhibitory potential of DA D3 agonism in relation to DA D1 stimulation of TD. The fact that the DA D3 antagonist, on the other hand, did not significantly exacerbate the DA D1 agonist induced dyskinesia, might have been due to a ceiling effect occurring at the concomitant dose of 0.3 mg/kg SKF 81927. Indeed, no further worsening of dyskinesia was observed when the dose of SKF 81287 was increased from 0.3 to 0.6 mg/kg.

Regarding effects on baseline dyskinesia, we found that the DA D3 antagonist induced a significant increase in dyskinesia compared to placebo, while inducing no other movement disorders or other behavioral actions. The DA D3 agonists, however, did not significantly decrease baseline TD. This apparent lack of effect may have been due to the relatively low level of baseline TD. Thus, pramipexole, at all doses, did reduce dyskinesia in the three most severely affected animals. As to the effect of CIS-8-OH-PBZI, the emesis occurring at the tested dose (10 mg/kg) makes evaluation of this dose given alone unreliable. Unfortunately, there was not enough compound to test lower (possibly nonemetic) doses of CIS-8-OH-PBZI given alone (a further supply ceased due to Novo Nordisk's discontinuation of its CNS research program).

Considering the results as a whole, it does appear that the DA D3 receptor plays a role in dyskinesia, but that it may not be the only subtype of the DA D2 receptor family involved. Thus, viewing Figs. 2 and 3, it appears that the DA agonist, which was less selective as to DA D3 vs. DA D2 agonism, was more effective in inhibiting both DA D1 agonist induced exacerbation of dyskinesia and baseline dyskinesia. Consequently, the present results suggest the involvement of both DA D2 and D3 receptor subtypes in dyskinesia. Studies of DA D2 agonism are not currently possible due to the lack of DA D2 specific ligands.

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