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ORIGINAL PAPER

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Extrapyramidal side effects during chronic combined dopamine D1 and D2 antagonist treatment in Cebus apella monkeys

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Abstract Previous studies in non-human primates have shown that tolerance to dystonia occurs during chronic dopamine D1 (D1) but not D2 antagonism and induction/aggravation of oral dyskinesia (TD) during D2 but not D1 antagonism. We were therefore interested in determining the effects of combined chronic D1 + D2 antagonism on dystonia and dyskinesia. To this intent, 8 male Cebus apella monkeys were treated 10 weeks with gradually increasing doses of D1 antagonist (NNC 112) + a D2 antagonist (raclopride), followed by 2 weeks of treatment with the D2 antagonist alone. Due to previous neuroleptic exposure, 5 monkeys had TD and all were sensitized to dystonia. During the combined antagonist treatment, tolerance to dystonia occurred; the tolerance disappearing upon discontinuation of the D1 antagonist and continuation of the D2 antagonist alone. Parallel to these results, improvement of TD was seen during the combined antagonist treatment with worsening during the D2 antagonist alone. Both the combined antagonists and the D2 antagonist alone resulted in moderate/severe bradykinesia, with no tolerance. These findings indicate that supplementation of traditional D2 antagonism with a D1 antagonist would lessen the risk of dystonia and allow alleviation of preexisting TD, though parkinsonian side effects might still occur. The findings further indicate that separate dopaminergic mechanisms control dystonia/dyskinesia and parkinsonism.

Key words Dopamine D1 antagonist · Dopamine D2 antagonist · Dystonia · Dyskinesia · Parkinsonism · Extrapyramidal side effects · Monkey

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Introduction

Dopamine D1 (hereafter called D1) antagonists can upon acute treatment with sufficiently high doses induce a cataleptic/dystonic syndrome in non-human primates which is identical (in its manifestation) to that produced by D2 antagonists [1, 2]. The two syndromes are furthermore alike in that they both can be effectively counteracted by anticholinergics or D2 agonists [2]. There are differences however. Thus, D1 agonists have been found to counteract D1 antagonist but not D2 antagonist induced dystonia [2]. The most striking difference though is that during chronic treatment with D1 antagonists, marked tolerance is induced towards dystonia, whereas during chronic treatment with D2 antagonists no or only slight tolerance occurs [3–6].

As to other extrapyramidal side effects (EPS), another important difference between D1 and D2 antagonism is that, in animal models of tardive dyskinesia (TD), D1 antagonism appears to have no or only a minor risk of inducing, TD whereas D2 antagonism imposes a major risk [4, 5, 7–9]. Either type of antagonist can induce catalepsy in rodents or bradykinesia in non-human primates, implying a potential to result in parkinsonian side effects in humans [4, 5, 10–12].

The findings of this last mentioned study, Peacock et al., 1999 [12] strongly indicated that tolerance to dopamine D_1 antagonist induced dystonia could be conferred to dystonia induced by a dopamine D_2 antagonist, whereas results as to dyskinesia were mixed. Based upon the above considerations and in order to further elucidate the findings of study [12], the intents of the present investigation were 1) to examine whether tolerance to D1 antagonist induced dystonia could be conferred to D2 antagonist induced dystonia and 2) to determine the effects of combined D1 and D2 antagonism upon dyskinesia.

Methods

Animals

Eight adult male Cebus apella monkeys, previously exposed to chronic treatment with D2 and D1 antagonists were used. The monkeys have been described in Gerlach and Hansen [4, 12]. All of the monkeys were sensitized to dystonia and five had developed stable mild to moderate oral dyskinesia. The monkeys had been free of medication for a period of 3 months prior to the investigation. During the investigation, the monkeys were individually housed in separate cages in a 12-h light/dark cycle, temperature- and humidity-controlled environment.

The animals were treated in accord with the Danish Animal Ethics Committee's guidelines for use of laboratory animals.

Drugs and design

The drugs used were the D1 antagonist, NNC 112 (Novo Nordisk) and the D2 antagonist, raclopride (Astra). These were freshly prepared in saline solution and given by s.c. injection. Having, by acute injection, determined the minimum dose of each antagonist required to produce dystonia in each individual animal (median dose raclopride 0.018 mg/kg (total range 0.008–0.04 mg/kg); median dose NNC 112 0.05 mg/kg (total range 0.025–0.1 mg/kg)), a combination of the two antagonists was given daily for a period of 10 weeks. The combination began with 0.005 mg/kg of each antagonist and the dose of each was gradually increased in parallel at intervals of 3–4 days. If dystonia appeared, the dose of the D2 antagonist was decreased to the previous dose, not inducing dystonia. After the 10-week period of combined treatment, the D1 antagonist was discontinued and the D2 antagonist treatment was continued for another 2 weeks, starting at the endpoint dose from the period of combined treatment. The dose of the D2 antagonist was thereafter adjusted as necessary to prevent dystonia.

Before and after the chronic treatment trial, saline was given as placebo.

Table 1 Evaluation. The effects of the drugs were evaluated by means of the rating scales below: 0, not present; 1, mild (slightly more pronounced than normal); 2, moderate (behavior pronounced but discontinuous); 3, severe (behavior continuous)

Behavior rating scale		Score
Sedation	Degree of drowsiness/sleep ranging from awake to sleeping and cannot be awakened even by gross stimuli (e.g. hand clapping)	0–3
Diminished reactivity	Orientation or aggressiveness to objects or the observer in response to stimuli ranging from normal (e.g. responds to key jangling) to severely decreased (does not respond to shouts or hoots)	0–3
Bradykinesia	Degree of slow/stiffened movements ranging from normal, free movements to fixed, maintained postures	0–3
Dystonia	Degree of clonic movements of the head, neck, limbs and trunk ranging from no clonic movements to violent casting about in the cage	0–3

Evaluation

The monkeys were evaluated daily, non-blind (GJ), for sedation, diminished reactivity, bradykinesia and dystonia using a 4 point rating scale (Table 1). The results of these non-blind ratings were used to determine the dose adjustments described above. Once a week, the sessions were video recorded for subsequent blind evaluation, at time 0 (before injection) and for 30 min intervals for a period of 90 min (after injection) (the same intervals used for the non-blind ratings). Besides the aforementioned behavioral ratings, oral dyskinesia was rated by actual counts/90s. Only the blind ratings were used in the statistical analyses of the results.

Statistical analysis

The data were evaluated by means of Friedman's test followed by Wilcoxon's paired test for non-parametric data, when Friedman's test was significant. The accepted level of significance was $P < 0.05$, while the accepted level for a tendency was $P < 0.10$. All data are represented by median values over the first 90 min after injection for all 8 animals. The interquartile range is used as a measure of the spread, as the results cannot be expected to follow a Gaussian distribution. The interquartile range is the length of the interval containing the central 50% of observations.

Results

Dystonia

From Fig. 1, it can be seen that the maximum dose of raclopride which could be given alone by acute injection, without producing dystonia, before chronic treatment was

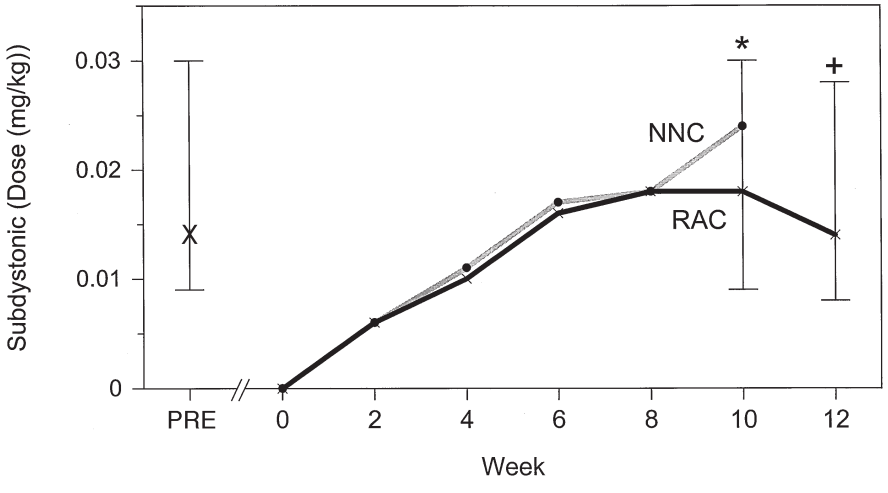

Fig. 1 Maximum dose of raclopride (RAC) not inducing dystonia during combined treatment with NNC 112 (NNC) and given alone. The curves show the median doses in mg/kg for all 8 monkeys for each time period, with the bars indicating the interquartile range. PRE = maximum acute subdystonic dose of raclopride prior to chronic combined treatment. *$P < 0.05$ as compared to PRE; + $P < 0.05$ end of combined treatment compared to raclopride alone

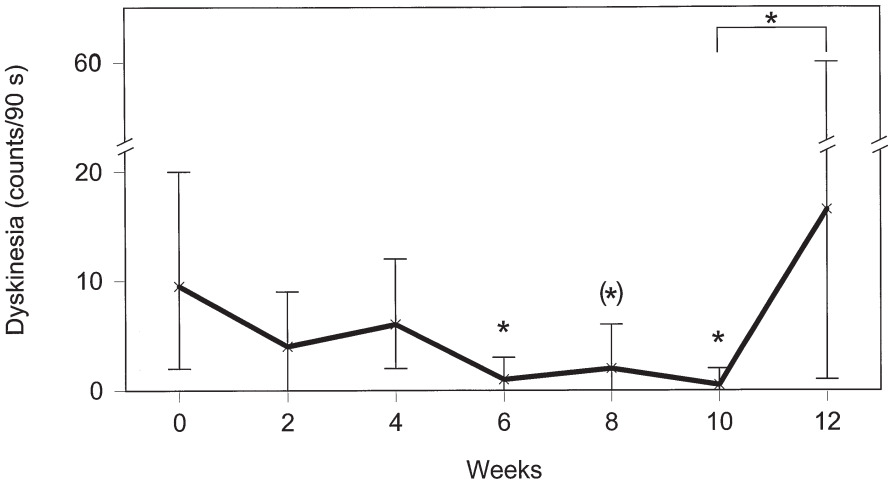

Fig. 2 Oral dyskinesia in counts/90s. The curve shows the median score for all 8 monkeys, the first 90 min after injection and the bars indicate the interquartile range. Week 0: placebo, weeks 2–10: NNC 112 + raclopride and 12: raclopride alone. (*)$P < 0.1$ and *$P < 0.05$ as compared to placebo and end of combined treatment compared to raclopride alone

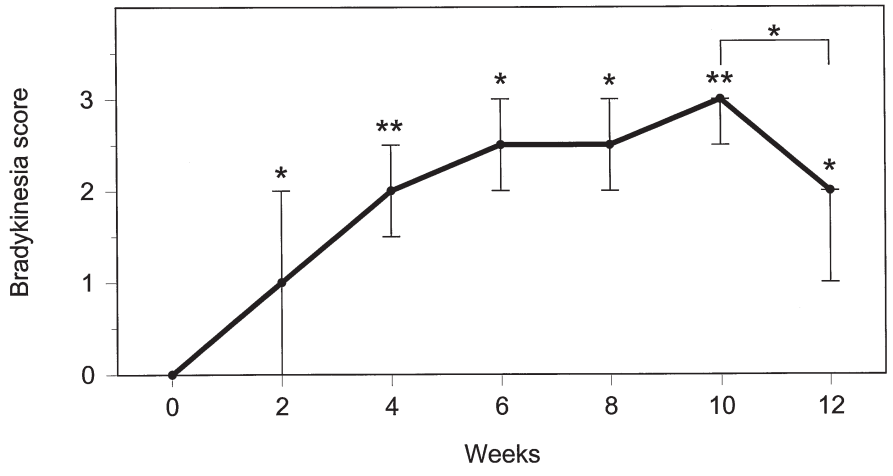

Fig. 3 Bradykinesia on a scale from 0–3. The curve shows the median score for all 8 monkeys, the first 90 min after injection and the bars indicate the interquartile range. Week 0: placebo, weeks 2–10: NNC 112 + raclopride and 12: raclopride alone. *$P < 0.05$ and **$P < 0.01$ as compared to placebo and end of combined treatment compared to raclopride alone

0.014 mg/kg (total range 0.008–0.03 mg/kg). The figure depicts the dose curves for each antagonist during the combined treatment and the following treatment with raclopride alone. It can be seen from the figure that during the combined treatment, the maximum subdystonic dose of raclopride significantly increased. At endpoint, a dose of raclopride, producing dystonia before combined treatment, could be given without dystonia occurring. After discontinuation of NNC 112, the dose of raclopride had to be significantly decreased in order to avoid dystonia.

Oral dyskinesia

From Fig. 2 it can be seen that the oral dyskinesia score fell during the chronic combined treatment, reaching significance as compared to placebo at week 6. After discontinuation of NNC 112, the dyskinesia score rose again, with an extreme exacerbation in three animals (score above 50 counts/90s). Dyskinesia was not provoked in the three animals without prior dyskinesia (score 0–3 counts/90s). There was no significant difference between the placebo socres at the beginning and end of the trial.

Bradykinesia

During both the combined treatment and treatment with raclopride alone, there was significant bradykinesia as compared to placebo (Fig. 3). From weeks 4–10, the bradykinesia scores did not significantly differ from one another. There was greater bradykinesia at the endpoint of the combined treatment phase than during the chronic raclopride phase.

Sedation and decreased reactivity

Both the combined treatment and raclopride alone resulted in significant sedation and decreased reactivity (Fig. 4 and 5). Though it might appear from the figures that effects as to the two behaviors were indistinguishible, there were differences. Thus, it was observed that there were animals which were obviously awake though nonreactive and vice versa, animals which slept yet remained reactive. Furthermore, there were differences in the course of development of the 2 behaviors. Thus, the animals were significantly more sedated from weeks 4–10 than week 2 and were more sedated at the endpoint of the combined

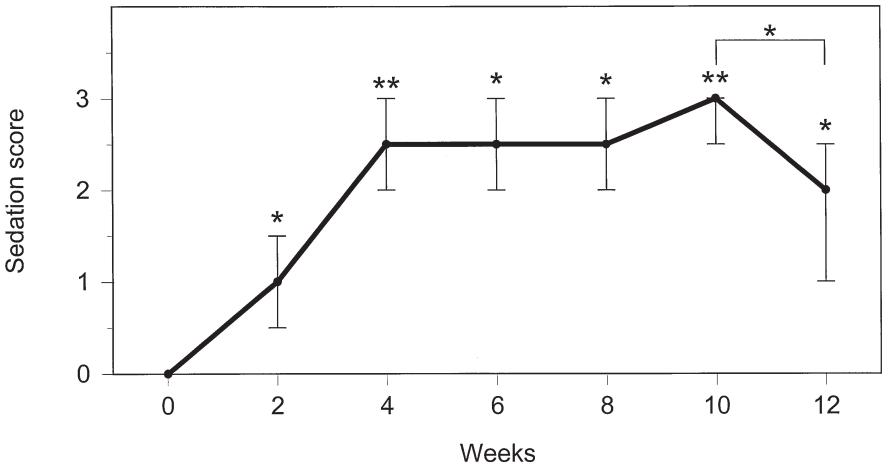

Fig. 4 Sedation on scale from 0–3. The curve shows the median score for all 8 monkeys, the first 90 min after injection and the bars indicate the interquartile range. Week 0: placebo, weeks 2–10: NNC 112 + raclopride and 12: raclopride alone. *$P < 0.05$ and **$P < 0.01$ as compared to placebo and end of combined treatment compared to raclopride alone

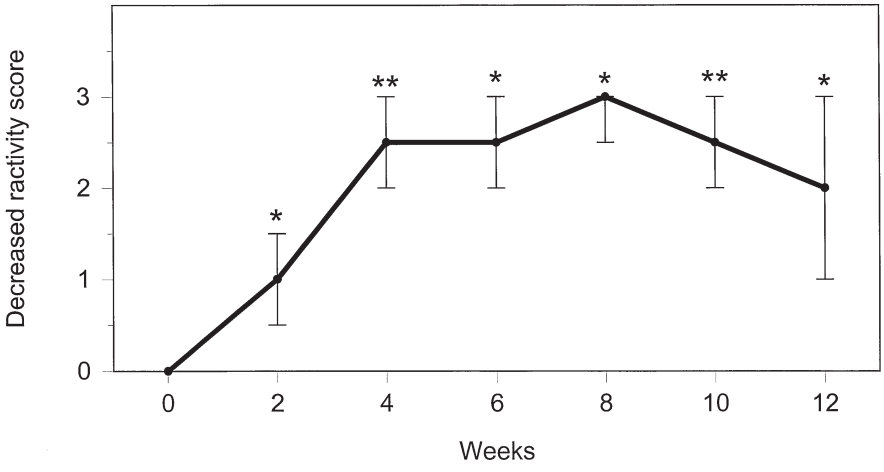

Fig. 5 Decreased reactivity on a scale from 0–3. The curve shows the median score for all 8 monkeys, the first 90 min after injection and the bars indicate the interquartile range. Week 0: placebo, weeks 2–10: NNC 112 + raclopride and 12: raclopride alone. *$P < 0.05$ and **$P < 0.01$ as compared to placebo

treatment than during raclopride alone, whereas there were no significant differences as to diminished reactivity from weeks 2–10 or between the combined treatment versus raclopride.

Discussion

One of the most outstanding aspects of D1 antagonism is that while acute high doses produce dystonia resembling D2 antagonist induced dystonia in character, manifest tolerance rapidly occurs during chronic D1 as opposed to D2 antagonism. Thus, it has been shown possible to increase the dose of a D1 antagonist 15-fold in non-naive and 100-fold in naive monkeys without inducing dystonia [3–5]. With D2 antagonists, it has not been found possible to continually increase the dose without dystonia occurring [3–5]. Modest tolerance, in the form of milder dystonia, has been found upon continued treatment with a fixed dystonic dose of a D2 antagonist, but dystonia nevertheless did still continue to occur [6].

In the present study, ongoing combination of a D1 antagonist with a D2 antagonist promoted slight though significant tolerance to dystonia, establishing the trend found in Peacock et al. 1999 [12]. That this result was not merely due to continued treatment with a dystonic dose of the D2 antagonist resulting in a mildening of dystonic symptoms is evidenced by the rapid return of dystonia upon discontinuation of the D1 antagonist. This latter finding correponds to the observation of Gerlach and Hansen [4] that tolerance to a D1 antagonist carried over to subsequent D2 antagonist treatment. Both the present study and that of Gerlach and Hansen [4] point to another aspect of D1 antagonist induced tolerance; that it is short lived after discontinuation, lasting only a few days to a week (i.e., the same time interval found required to develop tolerance to D1 antagonist induced dystonia). This rapid return of intolerance can also been seen upon reexposure to the same D1 antagonist after a short washout period following chronic treatment (own unpublished observations). Thus, though resensitization by the D2 antagonist might also have been a factor, such treatment is not a prerequisite to the return of intolerance.

The pharmacological mechanism leading to tolerance to dystonia during chronic D1 antagonism is not known. The time period of exposure required to develop and non-exposure to eliminate the tolerance may however help point to the mechanisms involved. Though an upregulation of D1 receptors has been demonstrated after chronic D1 antagonist treatment [13], this mechanism is an un-

likely explanation, based on the finding that a D1 agonist cannot counteract D2 antagonist induced dystonia [2]. Upregulation of D2 receptors is also an unlikely candidate as this occurs during chronic D2 antagonist treatment though no appreciable tolerance to dystonia ocurs (for reviews see [14 and 15]). In that treatment with a D2 agonist has however been shown to counteract dystonia induced by either a D1 or a D2 antagonist [1], it appears that the determining factor as to whether dystonia occurs or not is the level of D2 activation. Thus, a working hypothesis is that during chronic D1 antagonist exposure, increased efficiency of D2 activation develops, via promotion of cooperative coupling between D1 and D2 receptors [16].

In the present investigation we also found a significant suppression of oral dyskinesia during combined D1 and D2 antagonist treatment which disappeared after discontinuation of the D1 antagonist. There are several possible explanations for the suppression of dyskinesia during the combined treatment: 1) "masking" due to bradykinesia and/or sedation; 2) restoration of the balance between D1 and D2 activity (for review of the D1/D2 imbalance hypothesis of dyskinesia see [15] and 3) the development of tolerance parallel to tolerance to dystonia. Though bradykinesia/sedation may have played a role, these 2 behaviors reached a maximum before significant suppression of dyskinesia set in and furthermore, while there was also significant bradykinesia and sedation during raclopride alone, dyskinesia was not reduced during this treatment. Restoration of a balance between D1/D2 activity seems an unlikely explanation in that we have not found acute D1 antagonism to consistently reduce dyskinesia [2, 17]. The most plausible mechanism is induction of tolerance to acute dyskinetic effects parallel to tolerance to dystonia which would also explain the aggravation of dyskinesia in 3 animals during D2 antagonist treatment (when dystonia reappeared). This explanation is in line with the findings of other studies [2, 5].

As in other investigations we found either combined D1 and D2 antagonist treatment or D2 antagonist treatment alone, to result in significant bradykinesia, with no development of tolerance to this effect [4, 5, 12]. This implies that either treatment modality entails a risk of leading to parkinsonian side effects in the clinic. The finding further implies that the dopaminergic mechanisms involved in the induction of parkinsonism are separate from those involved in producing dystonia/dyskinesia, lending further support to earlier proposals that this might be the case [18–20].

Both chronic combined D1 and D2 antagonism and D2 antagonism alone resulted in sedation and diminishment of reactivity. As pointed out in the results section, these effects were distinghuishable, making it reasonable to assume that they are expressions of separate phenomena. A working hypothesis is that diminished reactivity is related to an antipsychotic potential.

In final summary, the present study points to a potential for combined D1 and D2 antagonism to provide lesser EPS liability in the clinic than traditional D2 antagonism alone. Thus, an element of D1 antagonism would be expected to reduce the risk of dystonia and to ameliorate preexisting TD. The extent of concomitant D1 blockade necessary to achieve this effect and whether such a combination would also reduce the potential to develop TD require further investigation.

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