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Levetiracetam improves choreic levodopa-induced dyskinesia in the MPTP-treated macaque

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Abstract

L-3,4 dihydroxyphenylalanine (levodopa)-induced dyskinesia in Parkinson's disease patients is characterized by a mixture of chorea and dystonia. Electrophysiological studies suggest that chorea is associated with abnormal synchronization of firing of basal ganglia neurons while dystonia is not. Levetiracetam is a novel anti-epileptic drug known to exhibit unique desynchronizing properties in contrast to other anti-epileptic drugs. We assessed the anti-dyskinetic efficacy of levetiracetam (13, 30 and 60 mg/kg, p.o.) administered in combination with an individually tailored dose of levodopa (Levodopa/carbidopa, 4:1 ratio, 19 ± 1.8 mg/kg, p.o.), in six dyskinetic 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned macaques. Levetiracetam (60 mg/kg) significantly reduced levodopa-induced chorea during the first hour post-treatment but had no effect on dystonia. Levetiracetam, at all doses tested, had no effect on the anti-parkinsonian action of levodopa. These results suggest that levetiracetam may provide a novel therapeutic approach specifically aimed at the choreic form of levodopa-induced dyskinesia.
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Keywords: Parkinson disease; Dystonia; Synchronization; Anti-epileptic drug

1. Introduction

Current treatments for Parkinson's disease are based largely on the dopamine precursor, L-3,4 dihydroxyphenylalanine (levodopa). Although initially successful, within 5 years of commencing levodopa treatment, up to 80% of patients will experience severe side effects (Rascol et al., 2000), such as loss of efficacy, marked unpredictable fluctuations in motor activity and dyskinesia (Bezard et al., 2001a). In levodopa-induced dyskinesia, there is a wide spectrum of phenomenology, including chorea, choreoathetosis, ballism, and dystonia (Bezard et al., 2001a).

Although the neural mechanisms underlying levodopa-induced dyskinesia in Parkinson's disease are far from clear, major advances have been made in recent years. On the one hand, there is evidence supporting the hypothesis that choreic and dystonic forms of dyskinesia are supported (or generated) by distinct pathophysiological mechanisms within the basal ganglia (Bezard et al., 2001a; Vitek, 2002). On the other hand, as foreshadowed in Filion's ground-breaking studies (Filion, 1979), Levodopa-induced dyskinesia is associated with an abnormally decreased firing frequency of basal ganglia output neurons at the level of the internal segment of the globus pallidus (Boraud et al., 2001; Hutchison et al., 1997). Moreover, both altered firing patterns (Boraud et al., 2001) and changes in the level of synchronization of globus pallidus pars internalis neurons have been sug-

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gested to contribute to dyskinesia genesis (Boraud et al., 2002). Interestingly, recordings performed in patients with generalized dystonia or hemiballism, a syndrome phenotypically related to levodopa-induced chorea in Parkinsonian patients, were suggestive of an increased degree of synchronization of globus pallidus pars internalis neurons in the patient with hemiballism that was not found in the dystonic patients (Vitek et al., 1999). Together these data suggest that choreiform levodopa-induced dyskinesia may be correlated to a persistent pathological synchronization that would not be fully normalized by the levodopa treatment (Boraud et al., 2002), i.e. a lack of desynchronization, while dystonic dyskinesia may not (Vitek, 2002).

We, thus, hypothesized that a drug able to reduce neuronal (hyper)synchronization may alleviate choreic Levodopa-induced dyskinesia whereas it would not affect the dystonic component of the levodopa induced side effects. Levetiracetam (Keppra®) is a novel anti-epileptic drug with proven efficacy as adjunctive therapy in patients with refractory partial epilepsy (Cereghino et al., 2000; Marson et al., 2001). Interestingly, it has been shown that inhibition of epileptiform activity by levetiracetam appears to contrast with other anti-epileptic drugs by involving a significant ability to reduce neuronal (hyper)synchronization (Klitgaard et al., 2003; Margineanu and Klitgaard, 2000). For this reason, levetiracetam appears to represent a powerful pharmacological tool for assessing the hypothesis that attenuation of synchronization may selectively decrease the severity of choreiform dyskinesia. This study, therefore, investigated the effect of levetiracetam on choreic and dystonic dyskinesia induced by chronic Levodopa treatment in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned macaque model of Parkinson's disease.

2. Materials and methods

2.1. Animals

Experiments were conducted on six female cynomolgus monkeys (*Macaca fascicularis*; Shared Animal Health, Beijing, China; age = 3–4 years; weight = 3–5 kg). Animals were housed in individual primate cages under controlled conditions of humidity (50 ± 5%), temperature (24 ± 1 °C), and light (12 h light/dark cycles, lights on 8:00 a.m.), food and water were available ad libitum, and animal care was supervised by veterinarians skilled in the healthcare and maintenance of nonhuman primates. Experiments were performed in accordance with European Communities Council Directive of November 24, 1986 (86/609/EEC) for care of laboratory animals. All efforts were made to minimise animal suffering and to use the minimum number of animals necessary to perform statistically valid analysis.

2.2. Induction of dyskinesia

Macaques were rendered parkinsonian by treatment with MPTP hydrochloride (Sigma, cumulative dose = 3.2 ± 0.2 mg/kg) as previously described (Bezard et al., 2001b). Once parkinsonism was stable, they were then treated with twice daily administration of Levodopa (Modopar®, Roche, Levodopa/carbidopa, ratio 4:1) for 4–5 months at an individually tailored dose designed to produce a full reversal of the parkinsonian condition (19 ± 1.8 mg/kg of levodopa). Over this period, animals developed consistent and reproducible dyskinesia that was characterized in detail for each of them. Apart from levodopa, animals were drug naïve at the time of the experiments.

2.3. Treatments

All drugs were administered in a single oral dose in a volume of 5 ml/kg via a syringe in the animal's home cage (Fox et al., 2002; Hill et al., in press). The animals were immediately transferred to an observation cage (dimensions—1.1 × 1.5 × 1.1 m) for behavioural assessment (Fox et al., 2002; Hill et al., in press). Four different treatments were employed; levodopa (19 ± 1.8 mg/kg) plus vehicle and levodopa (19 ± 1.8 mg/kg) in combination with levetiracetam (13, 30 and 60 mg/kg, respectively). The four different treatments were randomly tested three times in each animal. For each treatment, the median (range of movement, bradykinesia, posture and parkinsonian disability) or mean (activity counts and "on-time") of the three replicate values was used in statistical comparisons.

2.4. Behavioural assessment

A battery of behavioural tests was performed as previously described (Fox et al., 2002; Hill et al., in press). A quantitative assessment of locomotor activity using computer-based passive infrared activity monitors (Excalibur, modified by the Central Electronic Workshop, University of Manchester) was obtained every 5 min for the duration of the experiment (i.e. 6-h post-drug administration). Nonparametric measures based on range of movement, bradykinesia and posture scales were made, by post hoc analysis of video recordings by observers blinded to the treatment, in 10-min observation periods every 30 min throughout the duration of the experiment. The parkinsonian disability score was a combination of the range of movement, bradykinesia, posture and tremor scores according to the formula: (4 − range of movement) + bradykinesia + postural abnormality + tremor. Nonparametric measures of dyskinesia severity based on the following scale were made, by post hoc analysis of video recordings, in 10-min observation periods every 30 min throughout the duration of the experiment. Choreic (hyperkinetic, purposeless dance-like movements) and dystonic (sustained, ab-

normal muscle contractions) components of dyskinesia were rated separately with the following scale: 0 = Absent, 1 = Mild, fleeting, present less than 30% of the observation period, 2 = Moderate, not interfering with normal activity, present more than 30% of the observation period, 3 = Marked, at times interfering with normal activity, present less than 70% of the observation period, 4 = Severe, continuous, replacing normal activity, present more than 70% of the observation period. All scales have been validated for inter- and intra-variability and differences in rating were discussed regularly to eliminate observer idiosyncrasy (Taylor et al., 1994).

2.5. Graphical representation and analysis of data

"On-time" was defined as the total time, over the 6-h duration of the experiment, during which activity was above 50 counts per 5-min period. "On-time" data were plotted as mean ± S.E.M. Statistical analysis of cumulated activity data, cumulated over each 1-h period, and "on-time" data was carried out using a parametric repeated measures one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison's test (Graphpad Prism version 3). Parkinsonian disability, range of movement, chorea, dystonia, bradykinesia and postural abnormalities, cumulated over

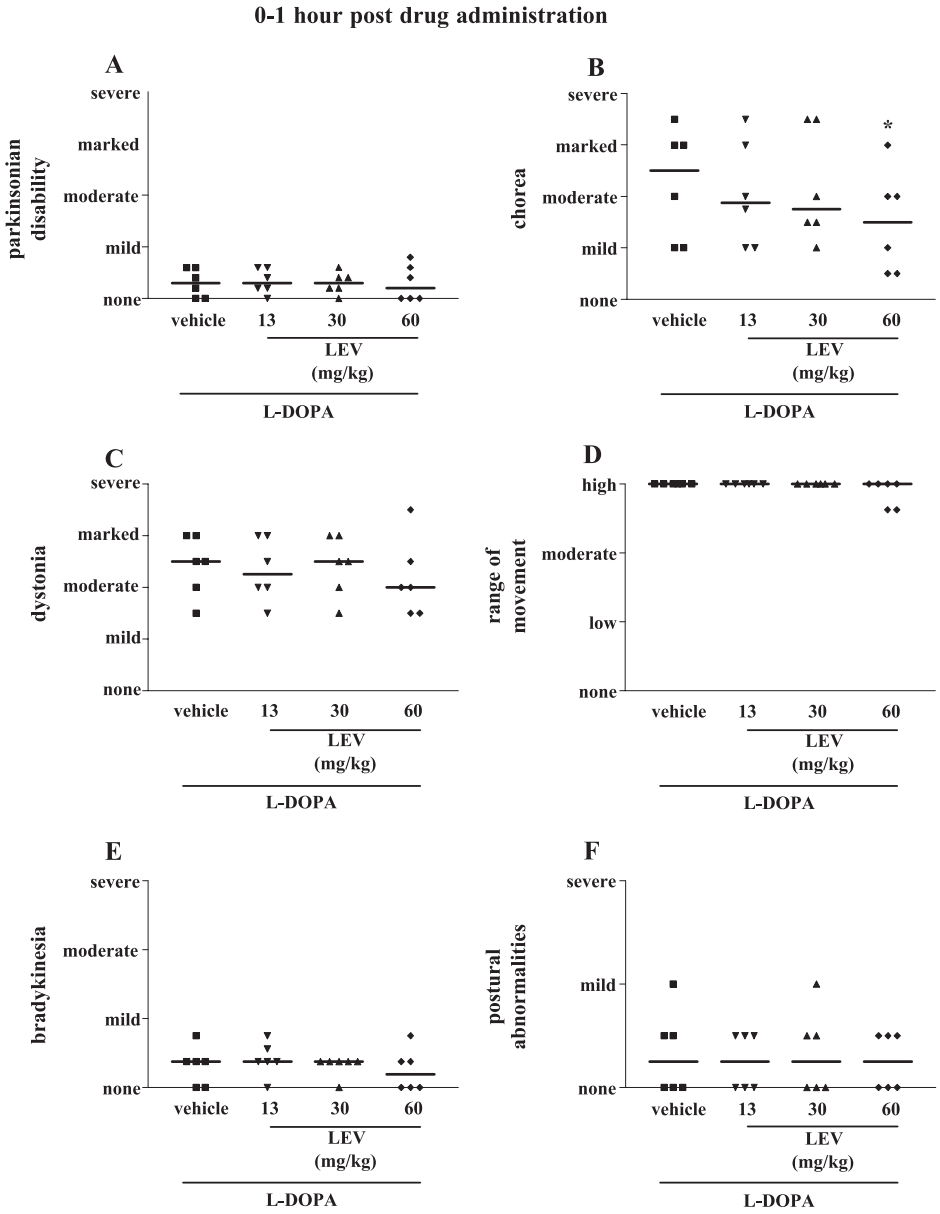

Fig. 1. The effect of levetiracetam (LEV; 13–60 mg/kg) in combination with levodopa (L-dopa) on (A) parkinsonian disability, (B) chorea, (C) dystonia, (D) range of movement, (E) bradykinesia and (F) postural abnormalities in the MPTP-lesioned macaque model of Parkinson's disease at 0–1 h post drug administration. Data are expressed as individual animal data with the median scores and was analysed with a Friedman test followed by Dunn's multiple comparison test. *$P < 0.05$, c.f. vehicle.

each 1-h period, were analysed with a nonparametric repeated measures one-way ANOVA (Friedman's test) followed by Dunn's multiple comparison test (Graphpad Prism version 3).

3. Results

3.1. Effect of levodopa monotherapy

Levodopa (76 ± 7 mg/kg) alone fully reversed parkinsonian symptoms. Following chronic Levodopa treatment, the alleviation of parkinsonism was invariably accompanied by dyskinesia. Dyskinesia was characterized by an idiosyncratic mixture of chorea and dystonia in all six animals. In all animals, both forms of dyskinesia reached the "marked" to "severe" level at their peak of intensity, i.e. during a 10-min observation period. During the first hour post-drug administration, animals were fully improved whatever the parkinsonian motor disability score considered (cumulated score over the 1-h period; Fig. 1A,D,E and F) but exhibited concomitantly "mild" to "severe" chorea (cumulated score over the 1-h period; Fig. 1B) and "moderate" to "marked" dystonia (cumulated score over the 1-h period; Fig. 1C). During 1–2 and 2–3 h periods, both choreic-like and dystonic like dyskinesia were also present (chorea: mild to marked and dystonia: mild to moderate). "On-time", as derived from activity counts (Fig. 2A), was 114 ± 12 min following levodopa monotherapy (Fig. 2B).

3.2. Effect of levetiracetam in combination with levodopa

Levodopa/levetiracetam combination therapy (60 mg/kg) significantly reduced choreic dyskinesia during the first hour post drug administration ($P<0.05$; Fig. 1B) but had no significant effect on chorea at any other time period post drug administration, i.e. between 1–2 and 2–3 h (all $P>0.05$). At 13 and 30 mg/kg levetiracetam, a tendency to reduce dyskinesia did not reach significance during the first hour of treatment. Levodopa/levetiracetam combination therapy (13 and 30 mg/kg) had no significant effect on chorea at any other time period post drug administration, i.e. between 1–2 and 2–3 h (all $P>0.05$). Contrary to the finding with chorea, dystonic dyskinesia was not significantly improved by combination therapy with any dose of levetiracetam at any time period post drug administration (e.g., during the first hour, see Fig. 1C).

The effects of co-administration of levetiracetam (13–60 mg/kg) and levodopa on parkinsonian disability, range of movement, bradykinesia or postural abnormalities were not significantly different to levodopa alone at any time period post drug administration (all $P>0.05$; effect during first hour shown in Fig. 1A,D,E and F, respectively). The anti-parkinsonian action of levodopa was, thus, fully preserved. "On-time", as derived from activity scores (Fig. 2A), was not significantly different from Levodopa monotherapy with any dose of levetiracetam (all $P>0.05$; Fig. 2B).

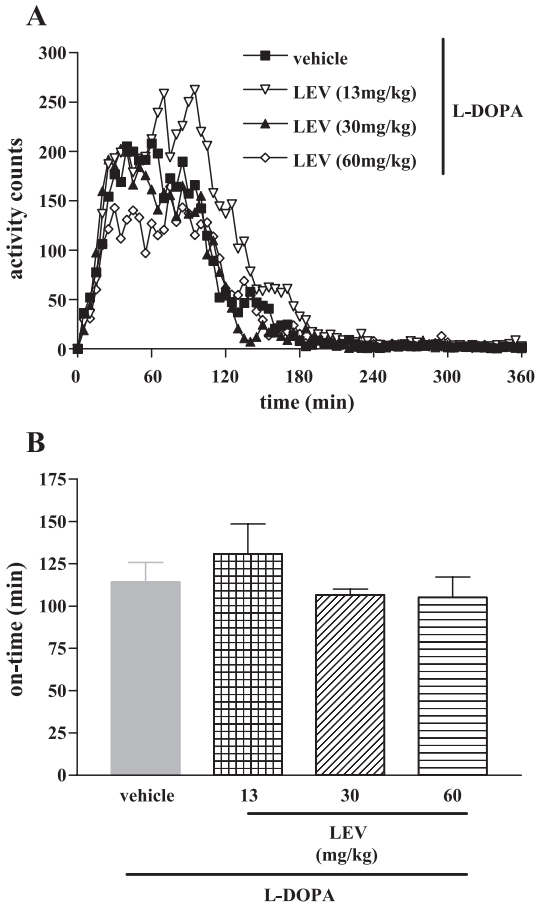

Fig. 2. The effect of levetiracetam (LEV; 13–60 mg/kg) in combination with levodopa (L-dopa) on (A) total activity counts and (B) on "on-time" (as derived from activity scores) in the MPTP-lesioned macaque model of Parkinson's disease. Data are expressed as the mean of six animals and analysed using repeated measures one-way ANOVA followed by Dunnett's multiple comparison test.

None of the few known side effects of levetiracetam in human patients such as somnolence were observed in the MPTP-treated macaques.

4. Discussion

The main finding of the present study is that levetiracetam, a novel anti-epileptic drug, which possesses unique desynchronizing properties in animal models of epilepsy, reduces choreic, but not dystonic, levodopa-induced dyskinesia in the MPTP-lesioned macaque model of Parkinson's disease.

The anti-choreic effect of levetiracetam was paralleled by a full preservation of the anti-parkinsonian efficacy of levodopa. A number of pharmacological approaches, dopaminergic or non-dopaminergic in nature, have been suggested to diminish the severity of dyskinesia (Bezard et al., 2001a). However, many are compromised by a negative effect on the anti-parkinsonian efficacy of the dopaminergic treatments or by side effects. The dose range of levetirace-

tam, i.e. 13–60 mg/kg, matches the recommended doses at add-on treatment of refractory epilepsy (Marson et al., 2001). Levetiracetam produces few side effects in human, and although somnolence, asthenia and dizziness may occur, there is no relationship between the dose used and these side effects (Harden, 2001). In keeping with these observations, no side effects were observed after levetiracetam treatment in the MPTP-treated macaques.

The anti-dyskinetic effect of levetiracetam lasted for 1 h. Levetiracetam is rapidly, and almost completely, absorbed after oral administration in human subjects (Harden, 2001). The half-life in man following oral administration of levetiracetam is 7 h but the rapid metabolism of the macaque would reduce its half-life. However, this appears not entirely to explain the transient anti-dyskinetic effect of levetiracetam. Another factor may relate to the animal species. Indeed, in a further experiment aimed at investigating the potential effect of levetiracetam in combination with amantadine, a noncompetitive *N*-methyl-D-aspartate receptor antagonist, the duration of action of levetiracetam alone was much longer in the marmoset (3 h) suggesting that the more transient effect observed in the present study may be linked to the species rather than to the levetiracetam itself (Hill et al., submitted). The observed anti-dyskinetic effect is however clinically relevant. The dyskinesia disability scale presently used is similar to the scale introduced into clinics and focuses on the issue of the disability caused by dyskinesia rather than the amount of dyskinesia (Brotchie and Fox, 1999; Pearce et al., 1995). Thus, the improvement corresponds to a dyskinetic activity that was marked, interfering with normal activity, and present for at least 70% of the observation period with L-dopa alone and that became mild, fleeting, and present for at least 30% of the observation period.

The findings of the present study highlight an interest in the possible molecular mechanisms of action of levetiracetam. In vitro and in vivo electrophysiological studies have shown that levetiracetam is distinct from other anti-epileptic drugs by its ability to inhibit neuronal (hyper)synchronization in animal models of epilepsy (Klitgaard et al., 2003; Margineanu and Klitgaard, 2002). The current concepts of the pathophysiology of Levodopa-induced dyskinesia stress both the excessively decreased firing frequency and alterations in firing pattern of globus pallidus pars internalis neurons as key functional targets, the restoration of which should provide clinical improvement (Boraud et al., 2001; Hutchison et al., 1997). In addition, abnormal synchronization of basal ganglia structures (Bergman et al., 1998), a characteristic feature of the parkinsonian syndrome, is not normalized by dopamine replacement therapy (Heimer et al., 2002). Of interest is the observation that globus pallidus pars internalis neurons show a greater degree of synchronization in a hemiballistic patient (Vitek et al., 1999), a condition comparable to the choreic component of dopamine-induced dyskinesia (Mitchell et al., 1985), while not in the patients with generalized dystonia (Vitek et al., 1999). These observations suggest that chorea is characterized by pathological synchronization of basal ganglia output structures while this is less marked for dystonia (Vitek, 2002). We can thus speculate that at least a part of the anti-choreic activity of levetiracetam may relate to desynchronization of abnormal neuronal firing patterns though this question deserves further investigation using electrophysiological recordings.

In conclusion, this study suggests that new non-dopaminergic treatment strategies, supposedly aimed at normalizing abnormal firing of basal ganglia output, may provide a novel therapeutic approach to choreic dopaminergic-induced dyskinesia. To what extent this confers a clinical potential to levetiracetam warrants further investigation.

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