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Brief Reports

Novel Antiepileptic Drug Levetiracetam Decreases Dyskinesia Elicited by L-Dopa and Ropinirole in the MPTP-Lesioned Marmoset

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Abstract: **Long-term dopamine replacement therapy of Parkinson's disease leads to the occurrence of dyskinesias. Altered firing patterns of neurons of the internal globus pallidus, involving a pathological synchronization/desynchronization process, may contribute significantly to the genesis of dyskinesia. Levetiracetam, an antiepileptic drug that counteracts neuronal (hyper)synchronization in animal models of epilepsy, was assessed in the MPTP-lesioned marmoset model of Parkinson's disease, after coadministration with (1) levodopa (L-dopa) or (2) ropinirole/L-dopa combination. Oral administration of levetiracetam (13–60 mg/kg) in combination with either L-dopa (12 mg/kg) alone or L-dopa (8 mg/kg)/ropinirole (1.25 mg/kg) treatments was associated with significantly less dyskinesia, in comparison to L-dopa monotherapy during the first hour after administration. Thus, new nondopaminergic treatment strategies targeting normalization of abnormal firing patterns in basal ganglia structures may prove useful as an adjunct to reduce dyskinesia induced by dopamine replacement therapy without affecting its antiparkinsonian action.** © 2003 Movement Disorder Society

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Long-term dopamine replacement therapy in Parkinson's disease leads to side effects such as dyskinesia.[1,2] Although dopamine receptor agonist therapy de novo has been shown to delay the onset of dyskinesia,[3] rescue therapy with levodopa (L-dopa) is usually required at some stage due to a reduction in efficacy over time. In addition, once patients have been "primed" with L-dopa, as is the case with the vast majority of Parkinson's patients, dopamine receptor agonists elicit dyskinesia, even when given as monotherapy. Thus, a pharmacological approach to the treatment of L-dopa–induced dyskinesia would be beneficial to the majority of Parkinson's disease patients.

The concept that L-dopa–induced dyskinesia is associated with an abnormally decreased firing frequency of the internal segment of the globus pallidus internalis (GPi) has been developed recently.[4,5] Both altered firing patterns and changes in the level of synchronization of GPi neurons have been suggested to impact on dyskinesia genesis.[6,7] In line with this concept, dyskinesia has been hypothesized to reflect a pathological synchronization/desynchronization process.[8] Of interest, levetiracetam (LEV; Keppra), a novel antiepileptic drug with proven efficacy as adjunctive therapy in adult patients with refractory partial epilepsy,[9] displays a unique ability to reduce neuronal (hyper)synchronization in animal models of epilepsy.[10,11]

For this reason, LEV appears to represent a potential pharmacological probe for testing the hypothesis that interference with synchronization processes may decrease the severity of dyskinesia. This study, therefore, investigated the effect of LEV on dyskinesia induced by L-dopa alone or by a combination of L-dopa and ropinirole, in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) -lesioned marmoset model of Parkinson's disease.

MATERIALS AND METHODS

Animals

Experiments were performed on seven adult common marmosets (*Callithrix jacchus*, 300–345 g, three male,

four female; BSU, University of Manchester) from a closed colony. This study was conducted in the United Kingdom in accordance with the requirements of The Animals Scientific Procedures Act, 1986. The animals were kept in controlled housing conditions, with constant temperature (25°C) and relative humidity (50%) and 12-hour light/dark cycles (8:00 AM lights on). The animals had free access to food (Masuri primate diet-E; Scientific Dietary Services, UK), fresh fruit supplements, and water. Marmosets were rendered parkinsonian by treatment with MPTP hydrochloride (Sigma, 2 mg/kg SC for 5 consecutive days) as previously described.[12–14] After stabilisation of the parkinsonian state, the animals were treated with Madopar dispersible (Roche; equivalent to 12 mg/kg L-dopa and 3 mg/kg benserazide) twice daily for 6 weeks as previously described.[12–14]

Drug Administration

All studies to assess the effects of LEV on dyskinesia were commenced between 9:00 and 10:00 AM. L-dopa alone (12 mg/kg as Madopar dispersible) or L-dopa/ropinirole combination (8 mg/kg as Madopar dispersible/1.25 mg/kg as Requip, respectively) was administered orally (5 ml/kg, dissolved in apple juice) with LEV (13–60 mg/kg) or vehicle, and the animals were placed in observation cages. The animals were not disturbed during the observation period, and the behavior was videotaped for 6 hours. A minimum of 48 hours was allowed between drug administrations to the individual animals.

Behavioural Assessments

A battery of behavioural tests was performed as previously described.[12–14] 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 minutes for the duration of the experiment. Nonparametric measures based on mobility, bradykinesia, and posture scales were made by post hoc analysis of video recordings by an observer blinded to the treatment in 10-minute observation periods every 30 minutes throughout the duration of the experiment. The Parkinsonian disability score was a combination of the mobility, bradykinesia, and posture scores according to the formula [18 + ((Bradykinesia * 3) + (Posture * 9)) − (Range of movement * 2)] to give a global parkinsonian disability rating. Nonparametric measures of dyskinesia severity based on the following scale were made, by post hoc analysis of video recordings, in 10-minute observation periods every 30 minutes throughout the duration of the experiment. Dyskinesias were rated as follows: 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, present more than 70% of the observation period, essentially replacing normal activity.

Graphical Representation and Analysis of Data

"On-time" was defined as the total time, over the 6-hour duration of the experiment, during which activity counts were above 50 counts per 5-minute period. "On-time" data were plotted as mean ± SEM. Statistical analysis of "on-time" was carried out using a parametric repeated measures one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison's test (*GraphPad Prism v. 3*; GraphPad, San Diego, CA). Data for parkinsonian disability and dyskinesia were cumulated for each 1-hour period and analysed with a nonparametric repeated measures one-way ANOVA (Friedman's test) followed by Dunn's multiple comparison test (Graphpad Prism, version 3).

RESULTS

Effect of L-Dopa Monotherapy

L-dopa (12 mg/kg) alone reversed parkinsonian symptoms (Fig. 1A). The alleviation of parkinsonian symptoms was accompanied by "severe" dyskinesia (Fig. 1B) that was characterized by a mixture of chorea and dystonia. "On-time," as derived from activity counts, was 99 ± 13 minutes (Fig. 1C).

Effect of Levetiracetam in Combination With L-Dopa

During the first hour after administration, dyskinesia was significantly less severe after the combined LEV/L-dopa treatment than with L-dopa alone ($P < 0.01$, $P < 0.05$, and $P < 0.01$ for 13 mg/kg, 30 mg/kg, and 60 mg/kg, respectively; Fig. 1B). At the same time, combination therapy was as effective in alleviating parkinsonian symptoms as L-dopa alone ($P > 0.05$ for 13 mg/kg, 30 mg/kg, and 60 mg/kg LEV; Fig. 1A). LEV had no significant effect on L-dopa-induced dyskinesia or antiparkinsonian action at any other time period after drug administration (all $P > 0.05$; data not shown). "On-time" was not significantly different between L-dopa monotherapy and LEV (13–60 mg/kg) combination therapy with L-dopa ($P > 0.05$ for 13 mg/kg, 30 mg/kg, and 60 mg/kg; Fig. 1C).

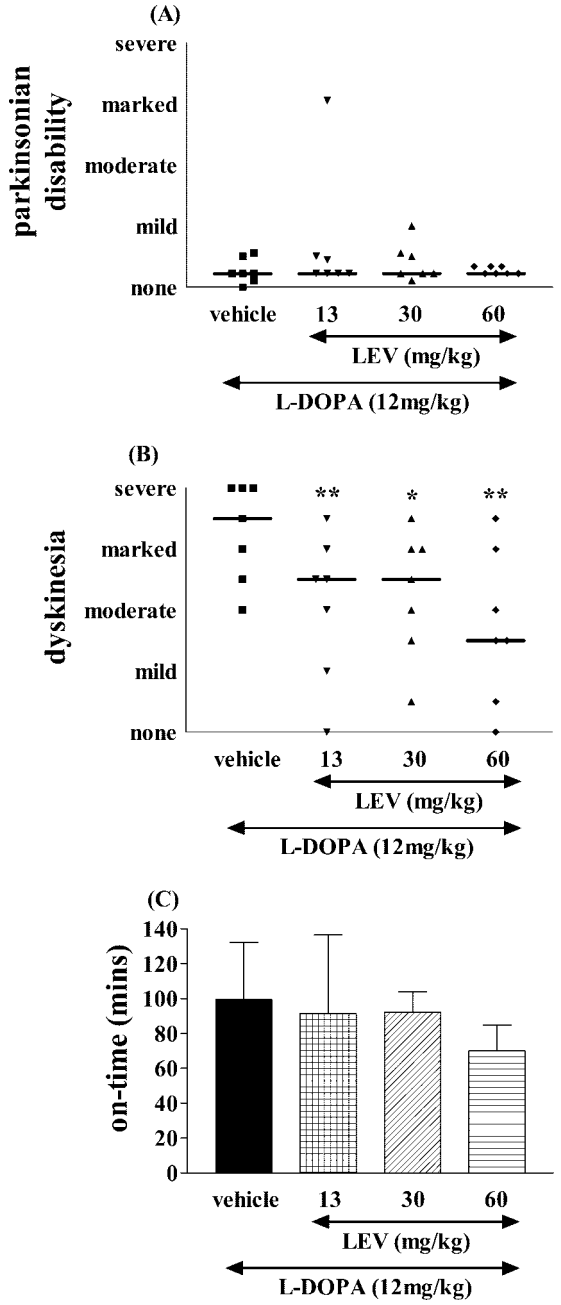

FIG. 1. The effect of levetiracetam (LEV, 13–60 mg/kg) in combination with levodopa (L-dopa, 12 mg/kg) on parkinsonian disability (**A**) and dyskinesia (**B**) at 0 to 1 hour after drug administration in the MPTP-lesioned marmoset model of Parkinson's disease. Individual animal data and the median scores are shown on the graphs. $*P < 0.05$, $**P < 0.01$ c.f. L-dopa + vehicle (Friedman's test followed by Dunn's multiple comparison's test). **C:** The effect of levetiracetam (13–60 mg/kg) in combination with L-dopa (12 mg/kg) on "on-time." Data are expressed as mean ± SEM; n = 7.

Effect of Ropinirole/L-Dopa Combination Therapy

L-dopa (8 mg/kg) in combination with ropinirole (1.25 mg/kg) reversed parkinsonian symptoms (Fig. 2A). "On-time" as derived from activity counts, was 273 ± 4 minutes after L-dopa/ropinirole combination therapy (Fig. 2C). The alleviation of parkinsonian symptoms was accompanied by dyskinesia (Fig. 2B) that was characterized by a mixture of chorea and dystonia. Dyskinesia was of a "marked" level during the first hour after administration of the ropinirole/L-dopa combination therapy (Fig. 2B), "severe" in the second hour, and then subsequently diminished to "marked" in the third and fourth hours.

Effect of Levetiracetam on Ropinirole/L-Dopa Combination Therapy

During the 0- to 1-hour time period, dyskinesia elicited by L-dopa and ropinirole in combination with LEV (60 mg/kg) was significantly less than that elicited by L-dopa/ropinirole alone ($P < 0.05$; Fig. 2B). However, no significant differences between dyskinesia elicited by LEV/L-dopa/ropinirole combination therapy and L-dopa/ropinirole were observed at any other time points for any dose of LEV (all $P > 0.05$; data not shown). LEV/L-dopa/ropinirole combination had no significant effect on disability score compared to L-dopa/ropinirole alone (all $P > 0.05$; Fig. 2A; data not shown for 2 to 4 hours after administration). LEV, administered at 13, 30, or 60 mg/kg in combination with L-dopa/ropinirole, had no effect on "on-time" (all $P > 0.05$; Fig. 2C).

DISCUSSION

The main finding of the present study was that LEV, a novel antiepileptic drug, reduces both L-dopa and L-dopa/ropinirole-induced dyskinesia in the MPTP-lesioned marmoset model of Parkinson's disease. The antidyskinetic effect of LEV was paralleled by a full preservation of the antiparkinsonian efficacy of both L-dopa alone and L-dopa/ropinirole combination. Several pharmacological approaches, dopaminergic or nondopaminergic in nature, have been suggested to diminish the severity of dyskinesia.[15] However, many are compromised by a negative effect on the antiparkinsonian efficacy of the dopaminergic treatments or by side effects. The dose range of LEV, i.e., 13 to 60 mg/kg, matches the recommended doses for add-on treatment of refractory epilepsy.[9] LEV 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.[16] In the present study, both clinical improvement and "on-

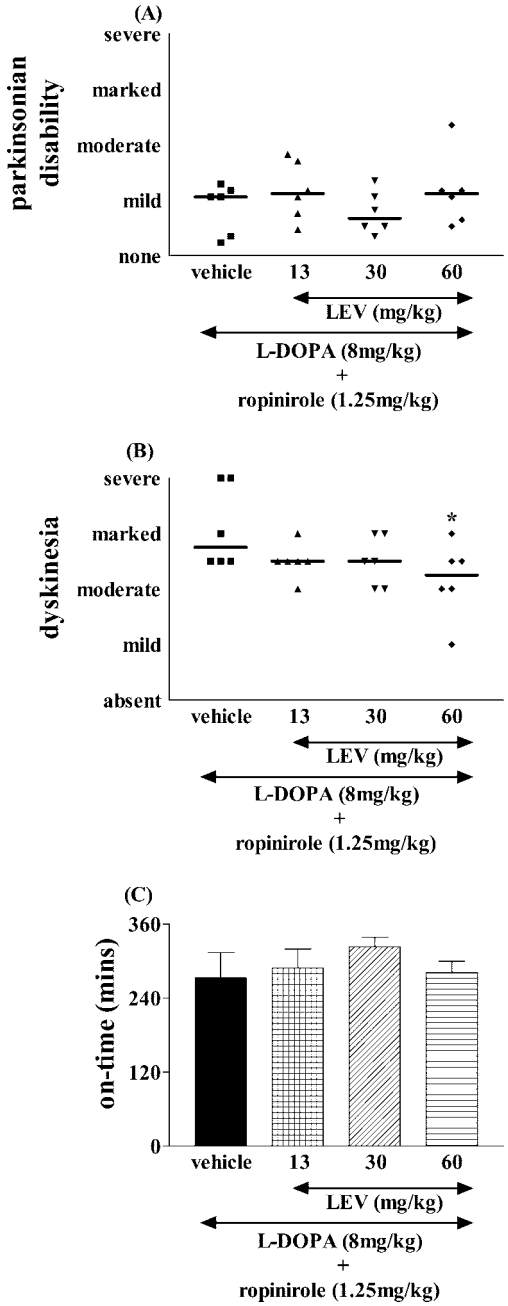

FIG. 2. The effect of levetiracetam (LEV, 13–60 mg/kg) in combination with levodopa (L-dopa, 8 mg/kg)/ropinirole (1.25 mg/kg) on parkinsonian disability (**A**) and dyskinesia (**B**) at 0 to 1 hour after drug administration in the MPTP-lesioned marmoset model of Parkinson's disease. Individual animal data and the median scores are shown on the graphs. $*P < 0.05$, c.f. L-dopa/ropinirole + vehicle (Friedman's test followed by Dunn's multiple comparison's test). **C:** The effect of levetiracetam (13–60 mg/kg) in combination with L-dopa (8 mg/kg)/ropinirole (1.25 mg/kg) on "on-time." Data are expressed as mean ± SEM; n = 6.

time" were preserved, whatever dopamimetic treatment was used, and no side effects were observed.

The antidyskinetic effect of LEV lasted for 1 hour whatever dopamimetic combination was used. LEV is rapidly and almost completely absorbed after oral administration in human subjects.[16] The half-life in man after oral administration of LEV is 7 hours, but the rapid metabolism of the marmoset would reduce its half-life. However, this appears not entirely to explain the transient antidyskinetic effect of LEV. Another factor may relate to the batch of animals. Indeed, in a further experiment aimed at investigating the potential effect of levetiracetam in combination with amantadine, the duration of action of LEV alone was much longer (3 hours), suggesting that the more transient effect observed in the present study may be linked to the level of lesioning of the animals rather than to the levetiracetam itself (data not shown, personal observations of M.H and E.B.). The observed antidyskinetic 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.[17,18] Thus, the improvement shown in Figure 1B 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 LEV. In vitro and in vivo electrophysiological studies have shown that LEV is distinct from other antiepileptic drugs by its ability to inhibit neuronal (hyper)synchronization in animal models of epilepsy.[10,19]This finding appears to correlate several recent electrophysiological studies supporting the concept that a decrease in the firing frequency of basal ganglia output in GPi is linked to clinical improvement with respect to the manifestation of dyskinesia.[5,20,21] Dyskinesia occurs when the firing frequency is excessively decreased[5,20,21] and the firing pattern is modified.[21] In addition, abnormal synchronization of basal ganglia structures,[22–24] a characteristic feature of the parkinsonian syndrome, is not normalized by dopamine replacement therapy.[7] We can speculate, thus, that at least a part of the antidyskinetic activity of LEV may relate to desynchronization of abnormal neuronal firing patterns. Further electrophysiological investigations addressing this question more directly are required.

In conclusion, this study is the first to suggest that new nondopaminergic treatment strategies that may normalize abnormal firing patterns of basal ganglia structures

may provide a novel treatment approach for dopaminergic-induced dyskinesia. To what extent this effect confers a clinical potential to LEV warrants further investigation.

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