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Repeated Administration of Piribedil Induces Less Dyskinesia Than L-Dopa in MPTP-Treated Common Marmosets: A Behavioural and Biochemical Investigation

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Abstract: Piribedil ([1-(3,4-methylenedioxybenzyl)-4-(2-pyrimidinyl)piperazine]; S 4200) is a dopamine agonist with equal affinity for D_2/D_3 dopamine receptors effective in treating Parkinson's disease as monotherapy or as an adjunct to levodopa (L-dopa). However, its ability to prime basal ganglia for the appearance of dyskinesia is unknown. We now report on the ability of repeated administration of piribedil to induce dyskinesia in drug naïve 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) -lesioned common marmosets compared with L-dopa and its actions on the direct and indirect striatal outflow pathways. Administration of piribedil (4.0–5.0 mg/kg orally) or L-dopa (12.5 mg/kg orally plus carbidopa 12.5 mg/kg orally twice daily) produced equivalent increases in locomotor activity and reversal of motor deficits over a 28-day study period. Administration of L-dopa resulted in the progressive development of marked dyskinesia over the period of study. In contrast, administration of piribedil produced a significantly lower degree and intensity of dyskinesia. Surprisingly, piribedil caused an increase in vigilance and alertness compared to L-dopa, which may relate to the recently discovered α_2-noradrenergic antagonist properties of piribedil. The behavioural differences between piribedil and L-dopa are reflected in the biochemical changes associated with the direct striatal output pathway. Administration of L-dopa or piribedil did not reverse the MPTP-induced up-regulation of preproenkephalin A mRNA in rostral or caudal areas of the putamen or caudate nucleus. In contrast, administration of either piribedil or L-dopa reversed the downregulation of preprotachykinin mRNA induced by MPTP in rostral and caudal striatum. L-dopa, but not Piribedil, reversed the decrease in preproenkephalin B mRNA produced by MPTP treatment. © 2002 Movement Disorder Society

Key words: piribedil; MPTP-treated primates; locomotor activity; vigilance; dyskinesia; Parkinson's disease

Levodopa (L-dopa, L-3,4-dihydroxyphenylalanine) remains the most commonly prescribed drug for the treatment of Parkinson's disease (PD). However, the development of abnormal involuntary movements (dyskinesia) is a common and dose-limiting side effect of its long-term use.[1–7] Despite more judicious use of L-dopa, dyskinesia still occurs in some 20 to 30% of PD patients after 5 to 10 years of treatment.[8–11] Once dyskinesia has been initiated by L-dopa, the movements persist and recur when other dopaminergic therapies are administered.[6,12] As a result, a strategic change in the early treatment of PD has occurred, such that dopamine agonists are commonly used as initial monotherapy to defer the use or to limit exposure to L-dopa and so prevent priming for dyskinesia. Piribedil is one such long-acting dopamine agonist drug[13] that shows selectivity for D_2/D_3 dopamine receptors.[14–19] It is effective in the treatment of PD when used either as monotherapy or in conjunction with L-dopa.[20–28] Prolonged clinical studies lasting up to 5 years with long-acting dopamine agonists such as bromocriptine, pergolide, ropinirole, pramipexole, or caber-

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goline have shown them to be effective in controlling motor symptoms of PD but with an incidence of dyskinesia lower than that occurring in patients treated with L-dopa.[29–34] Even when L-dopa was added to agonist monotherapy, the outcome with respect to dyskinesia was better than when using L-dopa alone.[30–33] In clinical use, piribedil is also thought to be associated with a lower incidence of involuntary movements, but no long-term study of its ability to induce dyskinesia when used as monotherapy in patient populations has yet been published.

The lower intensity of dyskinesia produced by dopamine agonists compared to L-dopa has been confirmed by studies undertaken in MPTP-lesioned primates. Repeated administration of long-acting dopamine agonists, including bromocriptine, pergolide, ropinirole, and cabergoline, consistently produces less dyskinesia than L-dopa in MPTP-treated monkeys that are otherwise drug naïve.[35–39] Comparison of short-acting and long-acting drugs has suggested that the development of dyskinesia may be linked to the duration of action of dopamine agonists. Long-acting compounds producing continuous dopaminergic stimulation appear less likely to prime for dyskinesia than short-acting drugs, which stimulate dopamine receptors in a pulsatile manner.[40–42] However, the repeated administration of equi-effective antiparkinsonian doses of apomorphine or pergolide to drug naïve MPTP-treated primates both caused less dyskinesia than L-dopa, despite marked differences in their duration of effect.[38] Nevertheless, all the studies undertaken in MPTP-treated primates suggest that dopamine agonists as a whole are less likely than L-dopa to prime the basal ganglia for dyskinesia induction. Indeed, the MPTP-treated primate appears to be predictive of drug action in man.[35,38,39,43–47]

The pathophysiology of dyskinesia is unclear but is thought to relate to an imbalance in the activity between the direct strio-GPi (internal globus pallidus) and indirect strio-GPe (external globus pallidus) striatal output pathways. The direct pathway arises from γ-aminobutyric acid (GABA)-containing striatal medium spiny neurons with projections to the GPi and substantia nigra pars reticulata (SNr). The direct GABAergic striatal output neurones coexpress excitatory dopamine D_1 receptors, dynorphin, and substance P (SP). The indirect pathway arises from striatal GABA-bearing medium spiny neurons projecting to the GPe. The indirect GABAergic striatal output neurones coexpress inhibitory dopamine D_2 receptors, enkephalin and adenosine A_{2a} receptors. The presence of neuromodulatory peptides in the direct and indirect pathways allows measurement of their mRNAs as an index of pathway function after drug manipulation. In both 6-OHDA (6-hydroxydopamine)-lesioned rodents and MPTP-treated primates, loss of the nigro-striatal pathway leads to an increase in the level of PPE-A (preproenkephalin A, preprodynorphin) mRNA in the indirect pathway. Conversely, a reduction of PPT (preprotachykinin) and PPE-B (preproenkephalin B) mRNA occurs in the direct pathway.[48–54] These findings form part of the consensus that the indirect pathway is overactive in PD and the direct pathway underactive.[36,55–57] From studies carried out in 6-OHDA–lesioned rats, MPTP-lesioned primates, and postmortem studies in patients with PD, L-dopa appears to regulate activity in the direct output pathway, as judged by increased levels of PPE-B mRNA and PPT mRNA. However, this does not occur in the indirect output pathway, as judged by persistently raised levels of PPE-A mRNA.[52,58–61] Lesioned-induced increases in PPE-A mRNA in the indirect pathway are not reversed by L-dopa but are either fully or partially reversed after treatment with dopamine agonist drugs, such as ropinirole and bromocriptine.[58,59,61,62] This has led to the concept that dyskinesia is associated with abnormalities of the indirect output pathway, although this is disputed.[63] However, the effects of piribedil on markers of striatal output have not so far been investigated.

In the current study, we sought to determine the effects of repeated administration of piribedil to drug-naïve, MPTP-treated common marmosets on dyskinesia induction compared with equi-effective doses of L-dopa. In addition, we examined the effects of piribedil on peptide markers of activity of the striatal output pathways to clarify why dopamine agonists are less able to prime for dyskinesia compared to L-dopa.

MATERIALS AND METHODS

Animals

Fifteen adult common marmosets (*Callithrix jacchus*) of either sex (weighing between 330 and 400 g) were used in the study. The animals were housed alone or in pairs under standard conditions at a temperature of 24 to 27°C and 50% relative humidity using a 12-hour light–dark cycle (light on from 8:00 AM to 8:00 PM). Animals were fed fresh fruits and "Mazuri" marmoset jelly once daily and had free access to food pellets and water. All procedures were carried out under Home Office Licence PPL 70/03563 of the Animals (Scientific Procedures) UK Act of 1986.

Administration of MPTP

MPTP hydrochloride (Research Biochemicals, MA) was dissolved in sterile 0.9% saline and administered once daily at 2.0 mg/kg subcutaneously for 5 days to

produce stable motor deficits. During MPTP treatment and throughout the following 4 to 8 weeks, the animals were hand fed until they had recovered enough motor function to feed themselves. During this period, the animals made a gradual recovery from the acute effects of MPTP. At the time of drug testing, however, all animals showed a marked reduction in basal locomotor activity, together with poor coordination, reduced checking movements of the head, loss of vocalisation, and abnormal posture of trunk and limbs.

Preparation and Administration of Drugs

L-Dopa methylester (Chiesi Farmaceutici, Italy) was dissolved in 10% sucrose solution (vehicle) and administered orally in a volume of 2 ml/kg. Piribedil monomethane sulphonate (Servier, France) was dissolved in 10% sucrose solution and administered orally by gavage in a volume of 2 ml/kg. Carbidopa (Merck, Sharp and Dohme, Rahway, NJ) and domperidone (Janssen, Belgium) were each administered orally as a suspension in 10% sucrose in a volume of 2 ml/kg. At the time of drug testing, animals were divided randomly into treatment groups (piribedil or L-dopa) according to baseline locomotor counts and motor disability scores to ensure that the two groups were equivalent from a locomotor point of view. Animals were dosed for 28 consecutive days as follows: Piribedil (n = 8) monotherapy was administered at 1 to 5 mg/kg (expressed as base) during the first 7 days of titration to achieve equivalent locomotor activity to that produced by L-dopa. Animals were treated with piribedil 1 mg/kg in days 1 to 2, rising to 3 mg/kg on days 3 to 4, and to 4 to 5 mg/kg on days 5 to 7. The dose was thereafter fixed at 4 to 5 mg/kg (expressed as base) once daily for the remaining 3 weeks of the study. Animals were pretreated daily with domperidone (2 mg/kg orally, in a volume of 2 ml/kg) 30 minutes before piribedil administration to prevent nausea and vomiting.[64] Additionally, animals were pretreated daily with carbidopa vehicle 45 to 60 minutes before administration of piribedil.

L-Dopa (n = 7) 12.5 mg/kg twice daily (expressed as base) plus carbidopa 12.5 mg/kg, were administered twice daily 45 to 60 minutes before L-dopa. Animals were also pretreated with domperidone vehicle 30 minutes before the first administration of L-dopa.

Assessment of Locomotor Activity

On each study day, animals were transferred from their home units and placed individually into activity units (50 cm wide × 60 cm long × 70 cm high) identical to those in which they were normally housed except for a clear plastic front that allowed greater visibility for observation. Each unit was fitted with eight horizontally orientated infrared photocell emitters, and their corresponding light detectors were arranged so as to permit maximal assessment of movement in all directions. Locomotor counts were measured as the number of light beam interruptions that occurred as the animals moved about. Animals were allowed a period of 45 to 60 minutes in the activity units to become acclimatized before determination of baseline or drugs-induced motility counts. Baseline motility counts were measured approximately 2 weeks before administration of piribedil or L-dopa, and animals were subsequently allocated to treatment groups such that basal motility counts in each group was equivalent. The monitor was started immediately after drug administration and motility counts were accumulated over 30-minute periods for 8 hours.[13,64,65]

Rating of Motor Disability

The following parameters of motor disability were assessed for each animal by observer rating: alertness (normal, 0; reduced, 1; sleepy, 2), reaction to stimuli (normal, 0; reduced, 1; slow, 2; absent, 3), head-checking movements (present, 0; reduced, 1; absent, 2), locomotor movements (normal, 0; bradykinesia/hyperkinesia, 1; akinesia/severe hyperkinesia, 2), posture (normal, 0; abnormal trunk, 1; abnormal limbs, +1; abnormal tail, +1; or grossly abnormal, 4), balance/coordination (normal, 0; impaired, 1; unstable, 2; spontaneous falls, 3), vocalisation (normal, 0; reduced, 1; absent, 2). The maximum disability score possible was 18, for which an animal was showing pronounced motor deficits. Animals used in this study had baseline disability scores (assessed approximately 60 minutes after being placed in the activity units and just before drug administration) of between 13 and 15. The animals were observer-rated for the last 10 minutes in each half-hour period for the following 300 minutes. Behavior was rated qualitatively to determine the presence or absence of grooming, stereotyped activity, retching/vomiting, oral movements, or other unusual motor activities. Assessments were undertaken by skilled behavioural pharmacologists (not blinded to drug treatments) with many years of experience in this field.[13,45–47,64–67]

Rating of Dyskinesia

Dyskinesia was described and assessed as follows: *Chorea*, rapid random flicking movements of the fore and hind limbs; *Athetosis*, sinuous writhing limb movements; *Dystonia*, sustained abnormal posturing. Dyskinesia was scored as follows: 0 = absent; 1 = mild, fleeting and rare dyskinetic postures and movements; 2 = moderate, more prominent abnormal movements, but

not significantly affecting normal behavior; 3 = marked, frequent and at times continuous dyskinesia affecting the normal pattern of activity; 4 = severe, virtually continuous dyskinetic activity, disabling to the animal and replacing normal behavior.[39,67] Dyskinesia was rated as chorea or dystonia for the last 10 minutes of each half-hour period for 300 minutes.[38,39,45–47,67,68]

Biochemical Analysis of Data

Tissue Preparation.

The day after the last drug administrations, brains were removed under terminal anesthesia with sodium pentobarbitone and rapidly frozen in isopentane at approximately −20°C and subsequently stored at −70°C. Coronal sections (20 μm) were cut using a Bright's cryostat at −20°C at A9.5 to 9.0 for caudal and A10.5 to 10.0 for rostral sections.[69] Adjacent sections were thermally mounted on gelatin-coated glass slides and stored at −70°C until assayed. Tissue from normal common marmosets and marmosets treated with MPTP but not receiving drug treatment previously prepared and stored for biochemical investigation was used in these studies to allow assessment of the effects of drug treatment on biochemical parameters.

In Situ Hybridisation Histochemistry.

In situ hybridization was carried out as previously described.[53,54] In brief, oligonucleotide probes complementary to bases 388-435 of human PPE-A cDNA,[70] and bases 205-252 of the human PPT cDNA[71] and bases of 418-462 of human PPE-B cDNA[72] were used. The probes were labeled using terminal deoxynucleotidyl transferase. The labeling reaction mixture consisted of 40 U of terminal deoxynucleotidyl transferase (Promega), 30 pmol of [^{35}S]dATP (1,300 Ci/mmol), 3.2 pmol of the probe, 8.0 μl of tailing buffer and distilled water to a 25.0-μl final volume. After a 1-hour incubation at 37°C, the reaction was stopped by the addition of 70.0 μl of 0.1 M TE buffer (10 mM Tris-HCL plus 1 mM EDTA buffer, pH 7.6) and 1.0 μl of tRNA (50.0 g/L). The oligonucleotide probes were separated by Nick column (Pharmacia Biotech, Sweden). Specific activity of the probes obtained was in the range of 8.0 to 11.0 × 10^3 Ci/mmol.

For hybridization, sections were warmed to room temperature and rinsed in phosphate buffered saline (PBS) and acetylated in freshly made 0.25% acetic anhydride in 0.1 M triethanolamine pH 8.0 for 10 minutes. Subsequently, they were dehydrated through ascending concentrations of ethanol (70, 80, 95, and 100%) and delipidated in chloroform for 2 × 5 minutes. Partially rehydrated (95% ethanol) sections were air-dried before hybridization. Labeled probes were diluted in hybridization buffer to a concentration of 5.0 to 7.0 × 10^6 dpm/ml (0.64 pmol/ml). The buffer contained 50% deionized formamide, 4 × saline-sodium-citrate buffer (SSC, 1× SSC containing 0.15 M NaCl and 0.15 M sodium citrate), 10% dextran sulphate, 1× Denhardt's solution (0.02% Ficoll, 0.02% polyvinyl pyrrolidone, 10.0 mg/ml bovine serum albumin, Sigma), 0.5 mg/ml boiled salmon sperm DNA, and 1 mM dithiothreitol (DTT). A Pap pen (Agar Scientific, UK) was used to make a boundary around the sections on the slides, and hybridization buffer (50 μl) was applied to each section. Sections were then allowed to incubate overnight in a humidified box at 37°C. After hybridization, slides were rinsed in 1× SSC and subsequently washed in 1× SSC at 55°C 4 × 15 minutes and then rinsed in 1× SSC and again for 2 × 30 minutes at room temperature. Finally, the slides were dipped in distilled water and blown dry.

The specificity of the probes was verified using brain sections pretreated with RNAse (50.0 μg/ml) at 37°C for 30 minutes before hybridization. In addition, sections were hybridized in the presence of a 50-fold excess of unlabelled probe to displace specific binding. In both cases, only evenly distributed background signal was detected. To generate autoradiograms, the slides along with ^{14}C standards (Amersham), were exposed to X-ray film (TM-βmax, Amersham, UK). The films were developed after 2 to 9 weeks of exposure time. For the biochemical data, autoradiograms were analyzed by computerized densitometry (MCID, Imaging Research, Canada). The ^{14}C standards were measured, plotted against known disintegrations per minute per mg, and converted to ^{35}S equivalence to generate a calibration curve.[73] For each section, putamen and caudate nucleus were outlined with a mouse-controlled cursor and the optical densities of the outlined areas were converted to nanocuries per μg wet weight of tissue from the standard curve and the values obtained were taken for statistical analysis. Nonspecific signal, as assessed from RNAse-treated sections, was subtracted from these values.

Statistical Analysis

In the behavioural studies, each group of animals was assessed three times per week for locomotor activity, disability, or dyskinesia. Overall statistical differences between piribedil or L-dopa–treated animals for total locomotor activity counts, total disability scores, total dyskinesia scores, and specific items of disability subscores (mean ± S.E.M.) were analyzed by the Kruskal-Wallis analysis of variance (ANOVA) test for nonparametric data. Cumulated dyskinesia scores on assessment days

for either piribedil or L-dopa expressed as median values were analyzed using Friedman's nonparametric ANOVA test. The level of significance was set at $P < 0.05$.

Biochemical data were analyzed by one-way ANOVA, followed by the post hoc Dunnett's test. Comparisons were made between naïve control animals or MPTP treatment alone (previously prepared and stored for biochemical investigation) and MPTP plus drug treatment groups (piribedil or L-dopa).

RESULTS

Locomotor Activity

Baseline motility counts assessed before administration of piribedil or L-dopa were low and consisted of intermittent bouts of slow motility with long periods of inactivity (mean cumulative locomotor counts ± S.E.M. over 8 hours ranged between 10 and 117 counts/30 minutes). During the first week of drug treatment, twice-daily administration of L-dopa (12.5 mg/kg plus carbidopa 12.5 mg/kg) produced an increase in locomotor activity with peak activity occurring after approximately 1 hour. Titration of the dose of piribedil (1–5 mg/kg; once daily) was used to produce an increase in locomotor activity, which was of a similar magnitude and duration to that observed after administration of L-dopa. Subsequently, the dose of piribedil was maintained at 4 to 5 mg/kg from week 2 to 4 to maintain equivalence to the actions of L-dopa. Over the following 3 weeks of treatment, the pattern of locomotor activity produced by piribedil and L-dopa treatment remained constant (Fig. 1A and B). Statistical analysis of total locomotor activity counts over weeks 2 to 4 showed no differences between piribedil and L-dopa treatment (Kruskal Wallis ANOVA, $H = 0.35$, $df = 1$, $P = 0.554$; Fig. 1B).

Motor Disability Ratings

Baseline disability scores in both groups showed a high degree of motor disability (such as akinesia/hypokinesia, hunched posture, and a lack of interest in the environment). In week 1 of drug treatment, twice-daily administration of L-dopa (12.5 mg/kg plus carbidopa 12.5 mg/kg) produced a marked reduction in disability scores. Animals receiving once daily administration of piribedil (1–5 mg/kg) also showed a marked reduction in disability scores from baseline values assessed before the start of drug administration. However, the reduction in disability scores induced by piribedil during the first week of treatment was less than that produced by L-dopa. During week 2, twice daily administration of L-dopa (12.5 mg/kg plus carbidopa 12.5 mg/kg) produced no further decrease in disability scores compared with week 1 (Fig. 2A). Furthermore, when the dose of piribedil was stabilized at 4 to 5 mg/kg, a further reduction in disability scores was observed. Statistical analysis of the total disability scores accumulated during week 2 and week 3 now showed no significant difference between piribedil and L-dopa treatments (Kruskal Wallis ANOVA, $H = 0.12$, $df = 1$, $P = 0.729$; Fig. 2B). During week 4, a significant increase in disability score in the L-dopa group compared to piribedil treatment, was linked to the marked occurrence of dyskinesia (Kruskal Wallis ANOVA, $H = 4.34$, $df = 1$, $P = 0.039$; Fig. 2B). During weeks 2 to 4 of drug treatment, there was no overall difference in the extent of the reversal of motor disability produced by piribedil or L-dopa. Statistical analysis of the total disability scores over weeks 2 to 4 showed no overall differences between piribedil and L-dopa treatment (Kruskal Wallis ANOVA, $H = 1.24$, $df = 1$, $P = 0.265$; Fig. 2B).

Analysis of specific items of disability subscores (alertness, head checking movements, posture, balance, reaction and motility) showed that both piribedil and L-dopa improved all aspects of disability with the exception of vocalisation (Fig. 2C; asterisks indicate $P < 0.05$, Kruskal Wallis ANOVA). However, piribedil produced a more marked effect on these subscore items and surprisingly was superior to L-dopa in items related to vigilance, for example alertness and head checking movements (Fig. 2C; asterisks indicate $P < 0.05$). Regarding alertness, although seven of eight animals receiving piribedil were rated "0" (normal), only four of seven in the L-dopa group were rated "0" in week 2. During weeks 3 and 4, all eight animals receiving piribedil were rated "0" for alertness, whereas only five of seven were rated "0" after treatment with L-dopa. No vomiting or signs of nausea (excessive salivation or retching) were observed in either piribedil or L-dopa–treated animals. However, intermittent marked grooming of the whole body and intense gnawing and licking of the wooden perches were frequently observed in both treatment groups. These behaviors are an exaggeration of normal behavior and are not stereotyped activities.

Assessment of Dyskinesia

Twice daily administration of L-dopa (12.5 mg/kg plus carbidopa 12.5 mg/kg) resulted in the appearance of increasingly marked dyskinesia during the first week of drug administration (Fig. 3A and B). In contrast, negligible dyskinesia was produced by piribedil (1.0–5.0 mg/kg). The overall difference in total dyskinesia between piribedil and L-dopa was highly significant (Kruskal Wallis ANOVA, $H = 10.50$, $df = 1$, $P = 0.001$; Fig. 3B). During week 2, there was a gradual increase in the incidence of dyskinesia after twice-daily administration

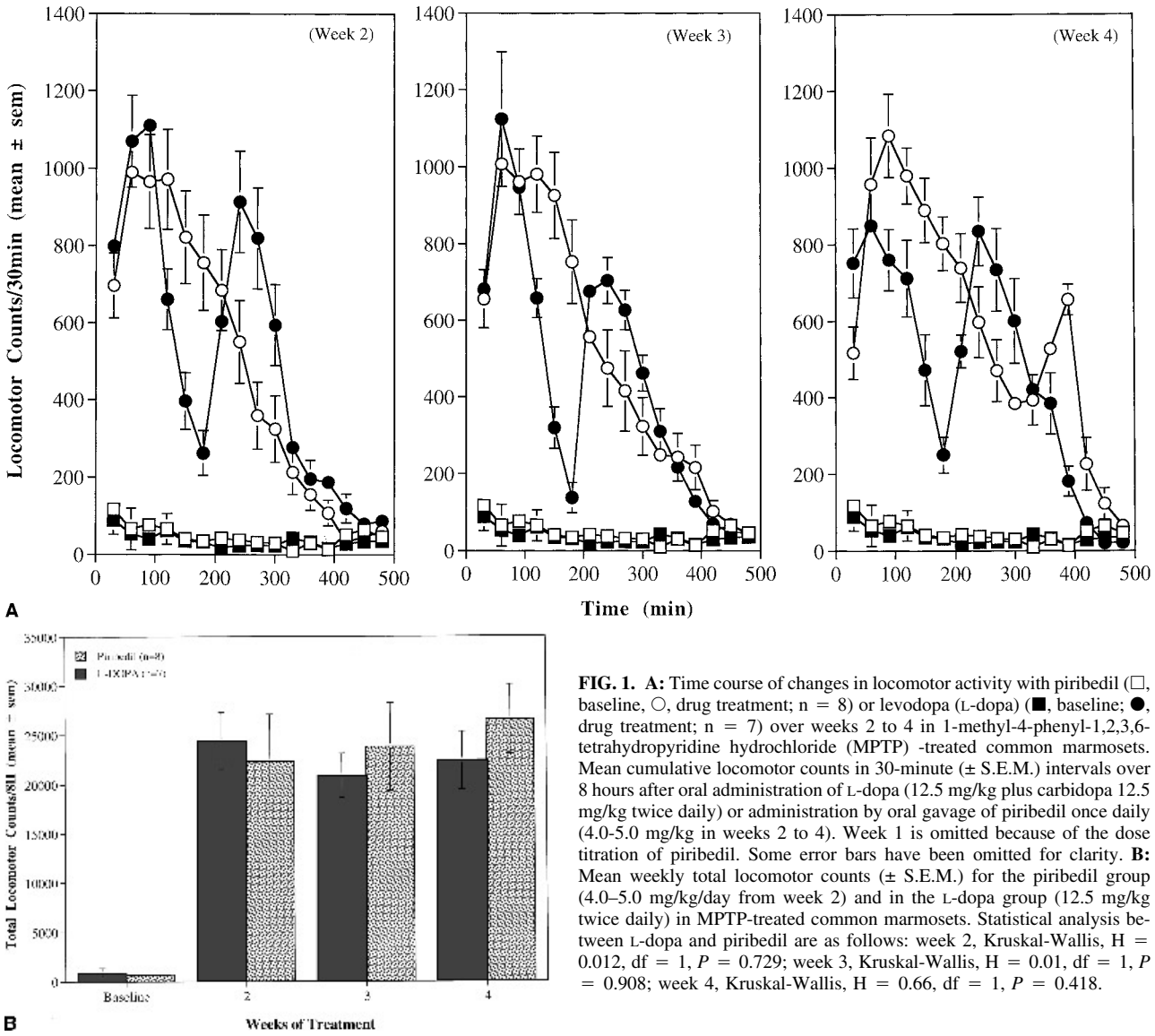

FIG. 1. A: Time course of changes in locomotor activity with piribedil (□, baseline, ○, drug treatment; n = 8) or levodopa (L-dopa) (■, baseline; ●, drug treatment; n = 7) over weeks 2 to 4 in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine hydrochloride (MPTP) -treated common marmosets. Mean cumulative locomotor counts in 30-minute (± S.E.M.) intervals over 8 hours after oral administration of L-dopa (12.5 mg/kg plus carbidopa 12.5 mg/kg twice daily) or administration by oral gavage of piribedil once daily (4.0-5.0 mg/kg in weeks 2 to 4). Week 1 is omitted because of the dose titration of piribedil. Some error bars have been omitted for clarity. **B:** Mean weekly total locomotor counts (± S.E.M.) for the piribedil group (4.0–5.0 mg/kg/day from week 2) and in the L-dopa group (12.5 mg/kg twice daily) in MPTP-treated common marmosets. Statistical analysis between L-dopa and piribedil are as follows: week 2, Kruskal-Wallis, H = 0.012, df = 1, $P = 0.729$; week 3, Kruskal-Wallis, H = 0.01, df = 1, $P = 0.908$; week 4, Kruskal-Wallis, H = 0.66, df = 1, $P = 0.418$.

of L-dopa (12.5 mg/kg plus carbidopa 12.5 mg/kg) or piribedil (4.0–5.0 mg/kg). However, the incidence and severity of dyskinesias was less severe in animals treated with piribedil compared with those receiving L-dopa (Fig. 3A and B). Analysis of total dyskinesia scores accumulated over the second week showed overall statistical significance between piribedil and L-dopa treatments (Kruskal Wallis ANOVA, H = 5.6, df = 1, $P = 0.021$; Fig. 3B).

Continued twice-daily administration of L-dopa (12.5 mg/kg plus carbidopa 12.5 mg/kg) during week 3 resulted in a progressive increase in dyskinesia when compared with week 2 (Fig. 3A). Administration of piribedil (4.0–5.0 mg/kg) also produced an increase in the manifestation of dyskinesia, although again this was less marked than that seen in the L-dopa group (Fig. 3A and B). Statistical analysis of the total dyskinesia scores accumulated over week 3 showed an overall significant difference between piribedil and L-dopa (Kruskal Wallis ANOVA, H = 4.34, df = 1, $P = 0.027$; $^*P < 0.05$; Fig. 3B). During week 4, marked dyskinetic movements became established in the animals receiving L-dopa. At this stage, the severity of dyskinesia hindered "normal" locomotor activity. In contrast, dyskinetic movements were not as marked in piribedil-treated animals. There was an overall statistical difference between piribedil

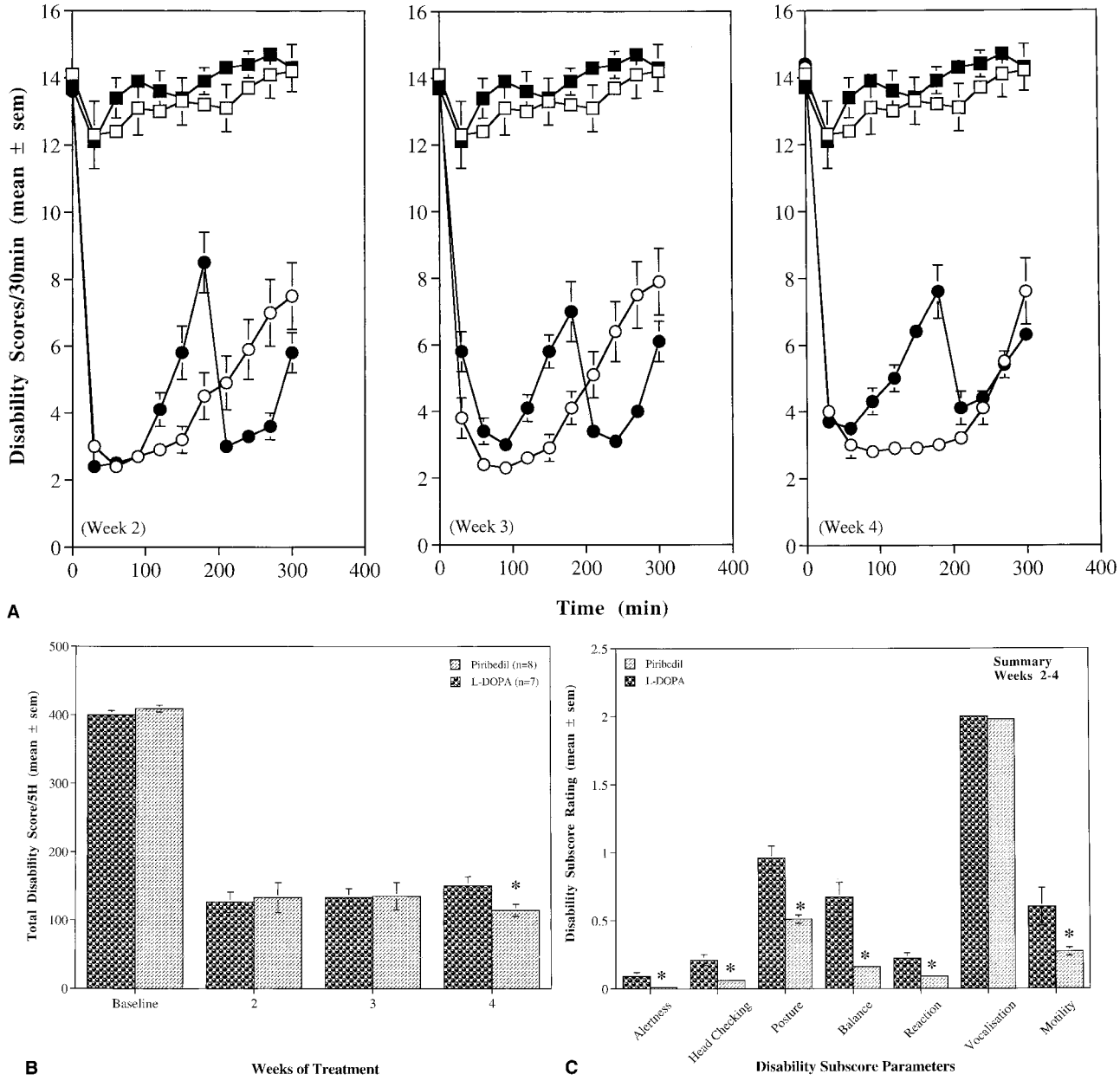

FIG. 2. **A:** Time course of changes in disability scores with piribedil (□, baseline; ○, drug treatment; n = 8) or levodopa (L-dopa) (■, baseline; ●, drug treatment; n = 7) during weeks 2 to 4 in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine hydrochloride (MPTP) -treated common marmosets. Mean cumulative disability scores in 30-minute (± S.E.M.) intervals over 5 hours after oral administration of L-dopa (12.5 mg/kg plus carbidopa 12.5 mg/kg, twice daily) or administration of piribedil by oral gavage (4.0–5.0 mg/kg per day). Week 1 is omitted because of the dose titration of piribedil. Some error bars have been omitted for clarity. **B:** Mean weekly total disability scores (± S.E.M.) in the piribedil group (4.0–5.0 mg/kg per day from week 2) and in the L-dopa group (12.5 mg/kg plus carbidopa 12.5 mg/kg, twice daily) in MPTP-treated common marmosets. Statistical analysis between the L-dopa and piribedil group are as follows: week 2, Kruskal-Wallis, H = 0.12, df = 1, $P = 0.729$; week 3, Kruskal-Wallis, H = 0.01, df = 1, $P = 0.908$; week 4, Kruskal-Wallis, H = 4.34, df = 1, $P = 0.039$. *$P < 0.05$ for the L-dopa group vs. piribedil group, Kruskal Wallis analysis of variance. **C:** Vigilance assessed as mean total index of alertness (A) or mean total index of head checking movements (B) (± S.E.M.) in the piribedil group (4–5 mg/kg per day from week 2) and in the L-dopa group (12.5 mg/kg plus carbidopa 12.5 mg/kg, twice daily) in MPTP-treated common marmosets.

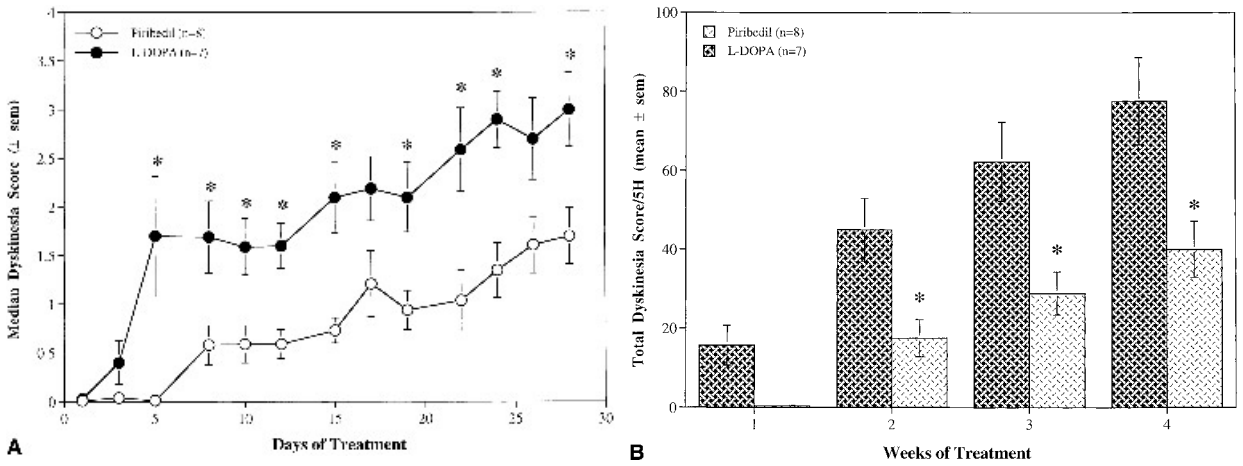

FIG. 3. A: Median dyskinesia scores in the piribedil group (○, n = 8) and the levodopa (L-dopa) group (●, n = 7) over 28 days in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine hydrochloride (MPTP) -treated common marmosets. L-Dopa (12.5 mg/kg plus carbidopa 12.5 mg/kg, twice daily) elicited a significant increase in dyskinesia ($P < 0.05$, Friedman's test, n = 7, F = 42.66). Although much less than L-dopa, piribedil (4.0–5.0 mg/kg per day from day 8) also elicited a significant increase in dyskinesia ($P < 0.05$, n = 8, F = 39.94). Each data point represents median dyskinesia score (ranges omitted for clarity) from absent = 0 to severe = 4. **B:** Mean weekly total dyskinesia scores (± S.E.M.) in the piribedil group (4–5 mg/kg per day from week 2) and in the L-dopa group (12.5 mg/kg plus carbidopa 12.5 mg/kg, twice daily) in MPTP-treated common marmosets. Statistical analysis between L-dopa and piribedil are as follows: week 2, Kruskal-Wallis, H = 5.36, df = 1, $P = 0.021$*; week 3, Kruskal-Wallis, H = 4.55, df = 1, $P = 0.037$*; week 4, Kruskal-Wallis, H = 4.83, df = 1, $P = 0.028$*.

and L-dopa treatment in favor of piribedil (Kruskal Wallis ANOVA, H = 4.83, df = 1, *$P = 0.028$; Fig. 3B).

Biochemical Analysis

PPE-A mRNA Expression

PPE-A mRNA was strongly expressed in cell clusters distributed evenly throughout the striatum in normal common marmosets (Fig. 4A). The expression in the putamen and caudate nucleus was higher in rostral areas when compared with caudal sections. There was also a decreasing gradient from the medial to the lateral areas within the caudate nucleus. In drug-naïve, MPTP-treated common marmosets, PPE-A mRNA labeling was increased throughout the caudate nucleus and putamen in both rostral and caudal sections when compared with normal animals (Dunnett's test, asterisks indicate $P < 0.01$, Fig. 4B and C). Treatment with piribedil or L-dopa did not alter the MPTP-induced increase of PPE-A mRNA expression in the caudate nucleus or putamen and in either rostral and caudal sections (Fig. 4B and C).

PPT mRNA Expression

In normal animals, PPT mRNA showed a patchy distribution in the caudate nucleus and putamen and the expression was less dense than for PPE-A mRNA (Fig. 5A). PPT mRNA levels in the caudate nucleus and putamen were decreased in drug-naïve MPTP-treated animals compared to normal controls (Dunnett's test, asterisks indicate $P < 0.05$, Fig. 5B and C). L-Dopa reversed the decrease in PPT mRNA levels such that, in both the caudate nucleus and putamen, there was an increase compared to values in control animals (Dunnett's test, single plus signs indicate $P < 0.01$, Fig. 5B and C). Similarly, piribedil reversed the MPTP-induced decrease in PPT mRNA in the caudate nucleus and putamen such that these again exceeded values in control animals and the levels found after L-dopa treatment (Dunnett's test, double plus signs indicate $P < 0.05$, Fig. 5B and C).

PPE-B mRNA Expression

There was a decrease in the levels of the PPE-B mRNA expression in both caudate nucleus and putamen in MPTP-lesioned compared to normal animals, although this decrease did not reach statistical significance. Treatment with piribedil had no effect on the MPTP-induced decrease in PPE-B mRNA expression. However, treatment with L-dopa reversed the MPTP-induced decrease to levels seen in normal animals (Fig. 6).

DISCUSSION

Dopamine agonists are currently used as monotherapy in the early treatment of PD to reduce the risk of dyskinesia induction.[30,33] Piribedil is one such centrally acting dopamine agonist that is effective in controlling the primary features of PD when used as monotherapy or in conjunction with L-dopa.[27,74] However, there has not previously been an assessment of its propensity to initiate involuntary movements when used as monotherapy. Therefore, this study was carried out to assess the dys-

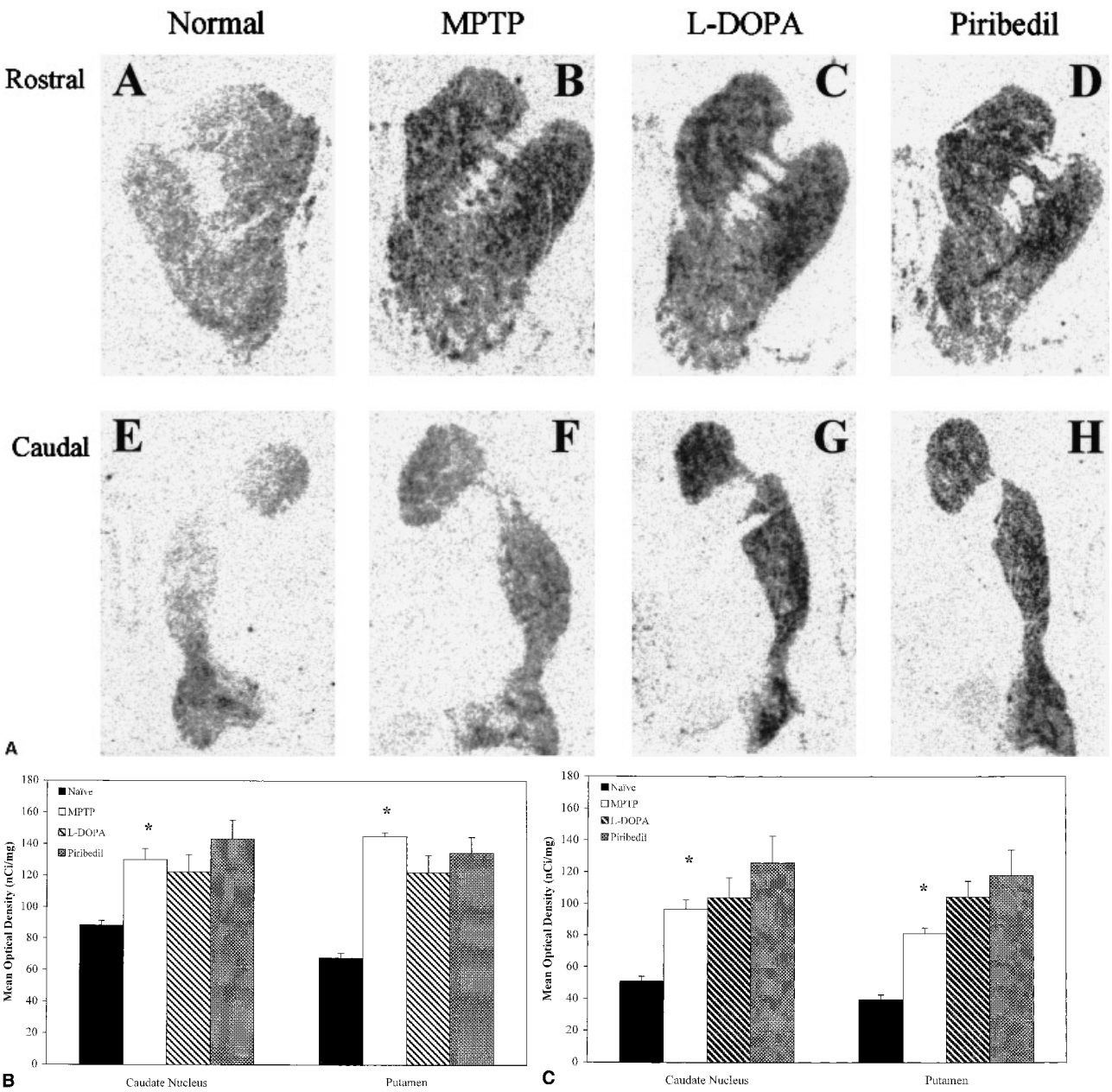

FIG. 4. A: Autoradiographic images are shown for in situ hybridization in the rostral level A11.5 (A–D) and images for the caudal level A9 to 9.5 are represented by E–H for preproenkephalin A (PPE-A) mRNAs. The sections of control (A and E), 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine hydrochloride (MPTP) -treated marmosets (B and F), with levodopa (L-dopa) (C and G), piribedil (D and H). **B:** Quantitative image analysis on the effects of L-dopa and piribedil on PPE-A mRNA expression in the rostral parts of the caudate nucleus and putamen of MPTP-treated common marmosets. Results are shown as mean ± S.E.M.; $*P < 0.01$ versus naïve control, one-way ANOVA followed by post hoc Dunnett's test. **C:** Quantitative image analysis of the effects of L-dopa and piribedil on PPE-A mRNA expression in the caudal parts of the caudate nucleus and putamen of MPTP-treated common marmosets. Results are shown as mean ± S.E.M.; $*P < 0.01$ versus naïve control, one-way ANOVA followed by post hoc Dunnett's test.

kinetic potential of repeated piribedil treatment in MPTP-treated, common marmosets compared with L-dopa. The results obtained underline that piribedil, compared to an equi-effective dose of L-dopa, is less likely to induce dyskinesia.

Several previous studies comparing the ability of L-dopa and dopamine agonists to induce dyskinesia on repeated administration to MPTP-treated monkeys have been undertaken. However, not all have been carried out under conditions allowing true comparisons of drug po-

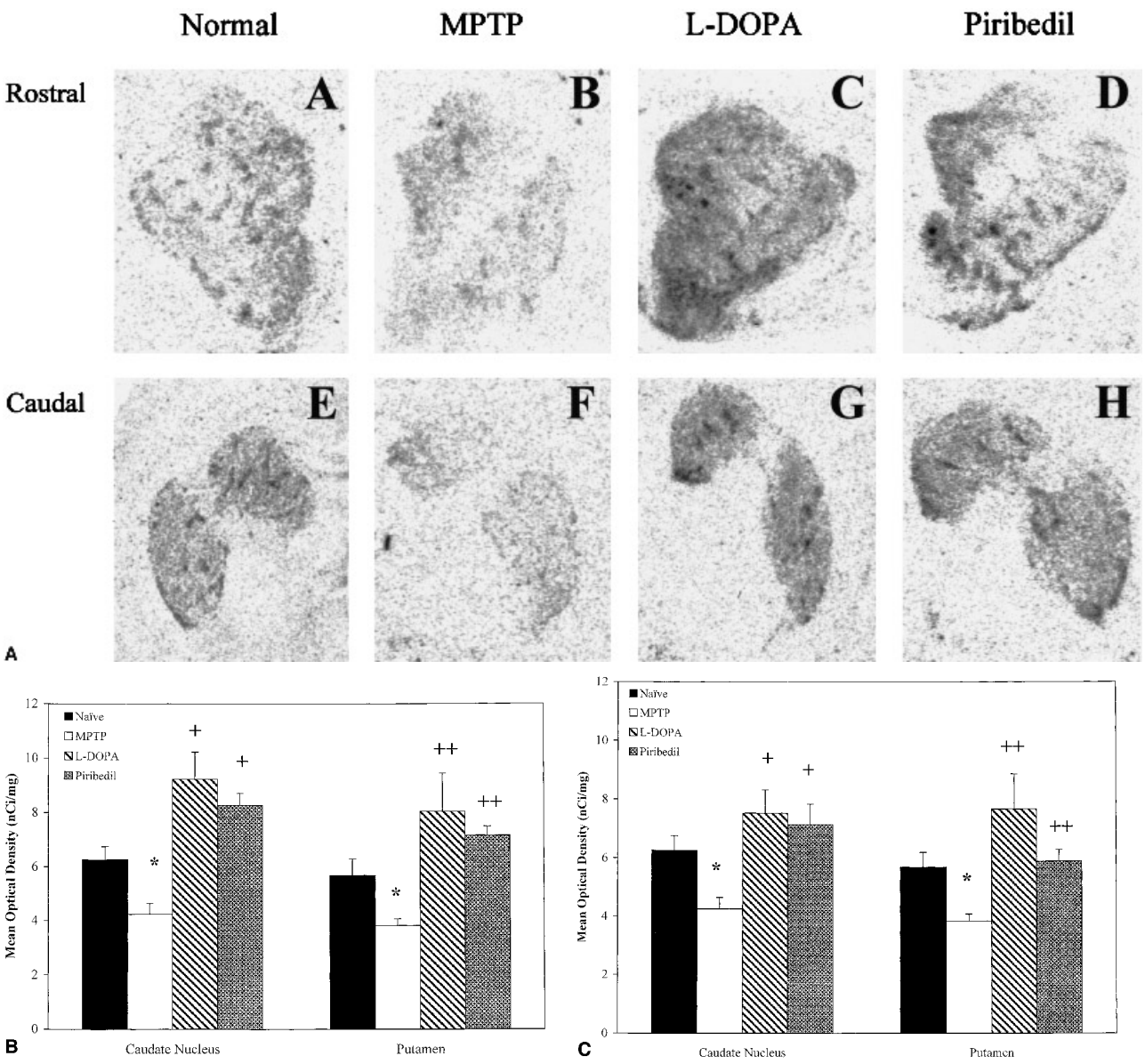

FIG. 5. A: Autoradiographic images are shown for in situ hybridization in the rostral level A11.5 (A–D) and images for the caudal level A9.0 to 9.5 are represented by (E–H) for preprotachykinin (PPT) mRNAs. The sections of control (A and E), 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine hydrochloride (MPTP) -treated common marmosets (B and F), with levodopa (L-dopa; C and G) and piribedil (D and H) are shown. The bar which represents μCi/mg wet tissue is shown for each image. **B:** Quantitative image analysis on the effects of L-dopa and piribedil on PPT mRNA expression in the rostral areas of the caudate nucleus and putamen of MPTP-treated common marmosets. Results are shown as mean ± S.E.M.; $^{*}P < 0.05$ versus naïve control; $^{+}P < 0.01$ versus MPTP treatment; $^{++}P < 0.05$ versus MPTP treatment, one-way ANOVA followed by post hoc Dunnett's test. **C:** Quantitative image analysis on the effects of L-dopa and piribedil on PPT mRNA expression in the caudal regions of the caudate nucleus and putamen of MPTP-treated common marmosets. Results are shown as mean ± S.E.M.; $^{*}P < 0.05$ versus naïve control; $^{+}P < 0.01$ versus MPTP treatment; $^{++}P < 0.05$ versus MPTP treatment, one-way ANOVA followed by post hoc Dunnett's test.

tential for dyskinesia induction. Important criteria for such studies, include ensuring that the extent of motor deficits is equal in different treatment groups and that the animals used are truly drug naïve in that no antiparkinsonian agents have been administered during recovery from the acute effects of MPTP treatment. It is also critical that the doses of agents used are equivalent in terms of ability to increase locomotor activity and reverse motor deficits. In this study, the animals were compared for basal motor activity and kept free of rescue medication before the start of piribedil or L-dopa treatment. Therefore, the first week of the study was used as a titration period during which the dose of piribedil was adjusted to produce a reversal of motor deficits equivalent to that

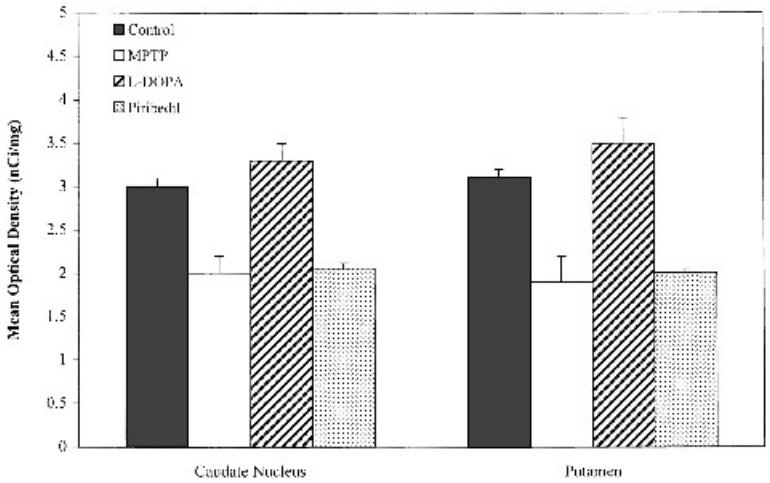

FIG. 6. Quantitative image analysis of the effects of levodopa (L-dopa) or piribedil on preproenkephalin B mRNA expression in the rostral parts of the caudate nucleus and putamen. MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine hydrochloride.

produced by a standard dose of L-dopa. We have previously used this dose of L-dopa in similar investigations and shown it to produce marked dyskinesia over the time course of such studies.[38,39,67] After the titration period and dose stabilization, both drug treatments produced equivalent motor responses during weeks 2 to 4 of the investigation.

The current experiments provided two unexpected responses to the administration of piribedil. First, the responsiveness of the animals to piribedil was much greater than we had seen in previous investigations involving this drug.[64] The major difference between studies was that this investigation was carried out in newly lesioned, drug-naïve, MPTP-treated animals as opposed to the use of animals lesioned with MPTP many months previously. The latter show some degree of motor recovery, which is accompanied by a small recovery in brain dopamine levels and a compensatory increase in dopamine turnover by remaining terminals.[75,76] Thus, it appears that piribedil is more effective in animals that have severe motor deficits and that have a greater impairment of dopaminergic function. In our extensive experience with MPTP-treated primates, we have not previously observed a similar increase in motor response to a dopaminergic agonist in newly treated MPTP animals. This phenomenon seems specific to piribedil and warrants further investigation of its pharmacology to discover the reason for its state-dependent actions.

Second, we found that piribedil produced an increase in vigilance and awareness resulting in enhanced responsiveness to the environment and external stimuli reported. We had previously observed this effect of piribedil, which is not seen with other dopamine agonists.[13,64] We have now quantified this effect and shown that piribedil produces a statistically greater increase in vigilance and awareness compared to L-dopa. This finding also warrants further exploration, because piribedil is claimed to be of benefit in psychobehavioral insufficiency (attention/interaction) resulting from cerebral aging in man.[77] In PD patients, piribedil has been reported to have an antidepressant effects unrelated to the improvement in parkinsonian symptoms.[25] Similarly, piribedil is reported to have beneficial effects in elderly patients with dopamine deficiencies and accompanying visuospatial processing disturbances.[78] The reason for this finding is not clear, but recently piribedil was shown to also act as an α_2 antagonist. The α_2 adrenoceptor antagonistic properties of piribedil may contribute to its functional actions, because in vivo piribedil blocked the hypnotic–sedative activity of the α_2 agonist, xylazine.[79] Other dopamine agonists such as pramipexole are α_2 agonists, and this action may be associated with the onset of sedation and excessive daytime somnolence.[80,81]

As expected, the repeated administration of L-dopa resulted in the rapid onset of dyskinesia that increased in intensity as the study progressed. This finding is in good agreement with other studies carried out in these laboratories and with the results obtained with L-dopa in other primate species.[35,38,39,44,82] The rapid onset of dyskinesia is probably a reflection of the extensive degree of nigral denervation in these animals, which lowers the threshold for dyskinesia induction. The situation in this primate model is akin to the rapid appearance of treatment complications, including dyskinesia, in MPTP-exposed parkinsonian drug addicts.[83–85] Piribedil administration also resulted in the appearance of dyskinesia, but these movements were much less marked in intensity than those produced by L-dopa. This finding is in line with our own

previous investigations with ropinirole and pergolide and with studies undertaken in other laboratories using a range of long-acting dopamine agonist drugs.[35,37–39] Because the MPTP-treated primate appears to be predictive of the ability of dopamine agonists to induce dyskinesia in PD patients, the results of this study suggest that early use of piribedil as monotherapy will have a low propensity to induce involuntary movements. The abnormal movements produced by piribedil were qualitatively similar in distribution (mild/severe leg dystonia, chorea, leg choreoathetosis) although less severe than those produced by L-dopa in agreement with previous reports for this species.[13,67] Indeed, the disruption of normal locomotor activity as a result of the severe dyskinesia produced by L-dopa by week 4 resulted in these animals being rated as more disabled than those receiving piribedil.

The mechanisms underlying the priming and expression of L-dopa induced dyskinesia remain unclear. Currently, L-dopa–induced dyskinesia is believed to relate to an imbalance between the direct and indirect striatal output pathways. After MPTP lesioning, the levels of PPE-A mRNA were increased in the striatum, while the levels of PPT and PPE-B mRNA were reduced. These findings are in line with data previously obtained from MPTP-treated primates[52,58,61] and 6-OHDA–lesioned rats.[49,51,53,86] The data are consistent with an increase in the activity of the indirect strio-GPe pathway and a decrease in the activity of the direct strio-GPi pathway after loss of the nigrostriatal input to the striatum. In this study, neither piribedil nor L-dopa had any effect on the increase in PPE-A mRNA expression in the rostral and caudal regions of the caudate nucleus and putamen in MPTP-treated common marmosets. Although this result is in agreement with previous reports from MPTP-treated primates,[51,61,87] it remains difficult to explain. Theoretically, it would be expected that dopamine replacement with L-dopa would reverse the change in PPE-A levels caused by nigral cell degeneration. Rather, L-dopa tends to increase PPE-A levels and in normal monkeys treated chronically with L-dopa, drug treatment elevates PPE-A levels above basal values.[54] The reason for this paradoxical effect is not known. It might relate to the onset of dyskinesia or it might reflect a more complex action of L-dopa that might explain why this drug is so effective in reversing motor symptoms of PD and in inducing dyskinesia compared to dopamine agonist compounds. These would seem to be reasonable explanations, because in other studies, chronic treatment of MPTP-treated common marmosets with bromocriptine or with ropinirole induced only mild dyskinesia but reversed the increase in PPE-A mRNA.[38,39,61] Whatever the reason, these results do not support the concept that the indirect pathway is underactive in L-dopa-induced dyskinesia in line with other findings.[57]

The actions of piribedil on the D_2 controlled indirect pathway were also unexpected given that the increase in PPE-A mRNA levels is responsive to other selective dopamine agonists with selective actions on D_2 and D_3 receptors, for example bromocriptine, pramipexole, and ropinirole.[19,49,50,59,61,62,88] It could be that the lack of change reflects the mild dyskinesia induced by piribedil or that it reflects a facet of the pharmacology of the drug, which remains to be described in line with the greater effectiveness of the compound in newly lesioned common marmosets. However, the results show a strong resemblance to the biochemical actions of L-dopa on the indirect pathway, which is not observed with other dopamine agonists but which may be relevant to the clinical activity of piribedil.

The decrease in levels of PPT and PPE-B mRNA as markers of the direct output pathway in MPTP-treated animals were reversed by L-dopa as previously described and consistent with normalization or overactivity of strio-GPi output consequential to L-dopa treatment. The decrease in PPT and PPE-B mRNA in the D_1 controlled direct pathway has previously been found to be resistant to the chronic administration of D_2/D_3 dopamine agonists.[61] Thus the ability of piribedil to reverse the decrease in PPT mRNA was totally unexpected. Reciprocal interactions are known to exist between D_1 and D_2 receptor systems, and previously a D_1 agonist has been shown to cause alterations in PPE-A levels in MPTP-treated primates.[62] However, if this were the case, then ropinirole and bromocriptine would have also been expected to reverse the decrease in PPT mRNA levels in MPTP-treated monkeys, but they did not.[61] Again, the pharmacological actions of piribedil can be questioned. The apparent actions of the drug on a D_1-mediated system suggest activity on this receptor system. Indeed, a metabolite of piribedil, namely S 584, can act as a D_1 agonist, because it stimulates dopamine-sensitive adenylate cyclase.[89–91] However, it has not been thought that S 584 has a sufficiently high affinity for D_1 receptors or that it is present in brain in sufficient amounts to contribute to the pharmacological actions of piribedil.[92,93] Even more puzzling was the failure of piribedil to induce changes in PPE-B mRNA. This marker of the direct output pathway was responsive to L-dopa administration in line with changes produced in PPT mRNA. Piribedil, in contrast, showed a differential effect on the mRNA for two peptide markers of the same pathway. We have no viable explanation for this disparity, but it serves to separate piribedil from L-dopa and from other long-acting

dopamine agonists acting on D_2/D_3 dopamine receptor populations.

In conclusion, piribedil is an effective antiparkinsonian agent that increases vigilance in MPTP-treated common marmosets and shows a lower propensity than L-dopa to initiate dyskinesia, and this feature is likely to reflect the frequency of involuntary movements in clinical use. The biochemical actions of piribedil set it apart from both L-dopa and dopamine agonist drugs. This and other features of its pharmacological profile warrant a further investigation of the mechanisms through which piribedil acts. The recently discovered α_2 antagonist properties of piribedil may be one of its features that deserves particular attention.

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