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Effects of Acute or Prolonged Administration of Cabergoline on Parkinsonism Induced by MPTP in Common Marmosets

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NOMOTO, M., S. KITA, S.-I. IWATA, S. KASEDA AND T. FUKUDA. *Effects of acute or prolonged administration of cabergoline on parkinsonism induced by MPTP in common marmosets.* PHARMACOL BIOCHEM BEHAV **59**(3) 717–721, 1998.—The effects of a single treatment or chronic administration of cabergoline (1-[(6-allylergolin-8β-yl)carbonyl]-1-[3-(dimethylamino)propyl]-3-ethyl-urea), a potent, long-lasting dopamine receptor agonist, on parkinsonism induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in common marmosets were studied. The administration of 0.2 mg/kg or a higer dose of cabergoline began to reverse parkinsonism-like symtoms 60 min after a subcutaneous injection, and showed steady and constant effects throughout the observation period. For prolonged administration, 0.2 mg/kg cabergoline was injected daily for 22 consecutive days. Locomotor activity in MPTP-treated animals increased until it reached its peak on the third day, then it gradually decreased. Akinesia scores, rating the quality of movements, were also improved, and the improvement was sustained up to the last day of chronic administration. None of the animals developed abnormal behaviors after either acute or chronic administration. These results suggest that cabergoline has long-acting effects in the marmoset model of parkinsonism, and that it will be a useful agent for the treatment of Parkinson's disease, particularly in cases with fluctuating motor disabilities. © 1998 Elsevier Science Inc.

Chronic administration Dopamine receptor agonist Cabergoline Parkinsonism Primate Common marmoset

CABERGOLINE (1-[(6-allylergolin-8β-yl)carbonyl]-1-[3-(dimethylamino)propyl]-3-ethyl-urea) is an ergoline derivative with potent, selective and long-lasting dopamine receptor agonistic activity that has shown high specificity and affinity for the dopamine D_2 receptor (2). In healthy humans or hyperprolactinemic patients, oral cabergoline had a potent prolactin-lowering effect, lasting 4–7 days (1,4). Administration of cabergoline reversed catalepsy in reserpine-treated rats (13). Stimulation of dopamine receptors by dopamine receptor agonists increased locomotor activity of normal animals and reversed akinesia in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated animals (16,19). In the present study, we examined the effects of the acute or chronic administration of cabergoline on parkinsonism induced by MPTP treatment in common marmosets.

METHOD

Animals

Sixteen adult common marmosets (*Callithrix jacchus*, 2–3 years old) of either sex (nine males and seven females) and weighing 250–360 g were employed. The animals were housed with free access to food and water in an air-conditioned room with temperature of 26°C and humidity of 50 ± 5% and maintained under a constant 12 L: 12 D cycle (lights on at

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0700 h). All behavioral experiments were carried out between 0900 and 1700 h. This study was approved by the Committee of Animal Experimentation, Faculty of Medicine, Kagoshima University.

MPTP Treatment

Ten animals were injected with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) at a dose of 2.5 mg/kg IV under ketamine anesthesia (22.5 mg/kg IM). When a treatment with MPTP did not rendered animals obviously akinetic, another injections of MPTP were given 10 days later. The different dosage regimens were employed to counteract the markedly different susceptibility of individual animals to MPTP. At the end of MPTP treatment, animals were allowed to recover for up to 8 weeks. Seven animals that showed prominent parkinsonism-like symtoms were used for studies. The remaining six animals received sterile saline injections, and four animals with body weight similar to MPTP-treated animals were employed as normal controls. MPTP was dissolved in sterile saline.

Behavioral Assessments

Each animal was removed from its home cage and placed in a metal grating cage with two wooden perches for the entire test period. The test cage (44 cm wide $\times$ 44 cm deep $\times$ 57 cm high) was moved with the animal in it to the test room, where a video recording of the animal's behavior was made. The animal was injected with vehicle or cabergoline in a volume of 1 ml/kg, and its spontaneous behavior was observed over the subsequent 320 min either in acute and prolonged administration. Seven MPTP-treated animals and four normal controls were injected with cabergoline at doses of 0, 0.05, 0.1, 0.2, and 0.4 mg/kg. Motor activity was measured by reviewing the videotape and counting the animal's movements across either the four base segments (22 $\times$ 22 cm) of the floor of the test cage, or across the four vertical segments (22 $\times$ 20 cm) between the floor and perches of the test cage. To evaluate drug-induced effects in the quality of motor behavior, an akinesia score was recorded 3 h after the injection of vehicle or cabergoline by an observer rating of video recordings according to the following rating scale (16): 0 = normal behavior; 1 = the animal appears quiet but shows a normal repertoire of movements; 2 = the animal can move freely, but is uncoordinated when making complicated movements, such as climbing down the cage wall; 3 = the animal makes fewer and slower movements and is obviously uncoordinated in executing complex movements, such as jumping up to a perch or moving on a perch; 4 = the animal makes few movements unless disturbed, and these are slow and limited to a small region of the cage; 5 = the animal is akinetic and does not move even when disturbed. During the observation period, the animal had free access to food and water. Observers scoring the animal behaviors had no access to information about the treatments.

Acute Administration

Each animal was allowed at least 30 min to become accustomed to the novel environment in the acute administration studies. Cabergoline or vehicle (0.067 M KH_2PO_4/Na_2HPO_4 solution) was injected subcutaneously at 1030 h. A period of more than 2 weeks separated individual administrations of either cabergoline or vehicle.

Prolonged Administration

Prior to repeated administration animals were set in the test room for 2 days for acclimatization. On the third day, behavior following vehicle injection was recorded and served as the prechronic treatment control behavior. Cabergoline was administered subcutaneously once a day (1030 h) for 22 consecutive days at a dose of 0.2 mg/kg. Animals were videotaped on the first, third, eighth, 15th, and 22nd day of cabergoline administration. Seven days after the last cabergoline injection, animals were videotaped to assess their behavior after a vehicle injection. Three weeks after cessation of daily cabergoline treatment, animals were administered cabergoline at a dose of 0.2 mg/kg and videotaped for behavioral assessment.

Drugs

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was purchased from Sigma Chemical Co. (St. Louis, MO); cabergoline (1-[(6-allylergolin-8β-yl)carbonyl]-1-[3-(dimethylamino)propyl]-3-ethyl-urea) was generously supplied by Kissei (Matsumoto, Japan). MPTP hydrochloride was dissolved in 0.9% sterile saline. Cabergoline was dissolved in 0.067 M KH_2PO_4, then adjusted to PH 6.0 by the adition of 0.067 M Na_2HPO_4. The final concentration of cabergoline was 0.05–0.4 mg/ml.

Statistical Analysis

Data were analyzed using the Kruskal–Wallis test (one-way ANOVA for nonparametric data) or the Friedman test (two-way ANOVA for nonparametric data), followed by the Mann–Whitney *U*-test. Results were considered statistically significant when *p*-values were less than 0.05.

RESULTS

Effects of MPTP Injection

Spontaneous locomotor activity that included moving around in the cage, and checking behavior such as swinging upper half of body without changing the sitting or standing place, or putting the head on either side, decreased markedly at the commencement of the study. Animals also exhibited slow and poorly coordinated behaviors, such as creeping down the wall of the cage rather than simply jumping from perch to floor. The akinesia score of MPTP-treated animals was 3.29 $\pm$ 0.18 (mean $\pm$ SEM). These animals also exhibited forearm tremor when extending their forelimbs without holding onto perches or cage bars. The akinesia score of control animals was 0.

Acute Effects of a Single Administration of Cabergoline

Single administrations of cabergoline at doses of 0.05 mg/kg did not reverse the parkinsonism of MPTP-treated common marmosets. At the dose of 0.1 mg/kg, one of seven MPTP-treated animals increased cheking behavior and spontaneous locomotor activity. At the dose of 0.2 mg/kg, checking behavior and spontaneous locomotor activity of animals started to increase around 1 h after administration in the all of seven MPTP-treated animals. Locomotor activity reached its peak 2 h after administration of 0.2 mg/kg cabergoline, and this level of activity was sustained during the entire 320 min observation period (Figs. 1 and 2). Animals made well-coordinated movements, for example, jumping to the floor or perch rather than climbing down or creeping up the wall. The effects of cabegoline on reversing akinesia were constant and continuous throughout the recording period. At the dose of 0.4 mg/

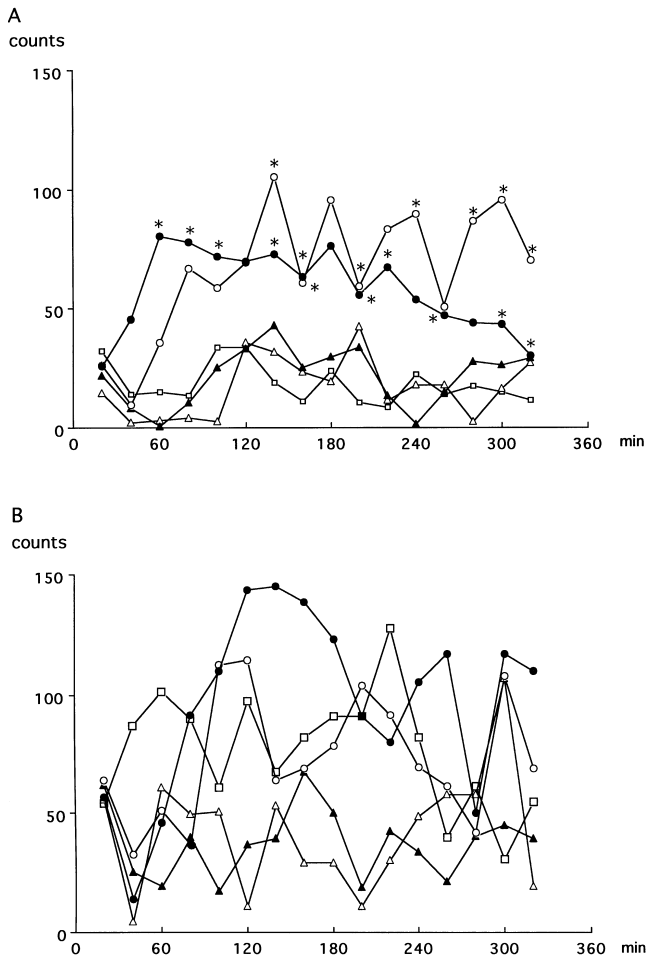

FIG. 1. Effects of acute administration of cabergoline on locomotor activity of MPTP-treated or normal control common marmosets. The values indicate mean locomotor activity of MPTP-treated animals (A, $n = 7$) or normal control animals (B, $n = 4$). Doses of cabergoline; □ 0 mg/kg, △0.05 mg/kg, ▲ 0.1 mg/kg, ○ 0.2 mg/kg, ● 0.4 mg/kg. *$p <$ 0.05 vs. MPTP-treated or normal control animals (0 mg/kg), Friedman test followed by Wilcoxon matched-pairs signed-ranks test.

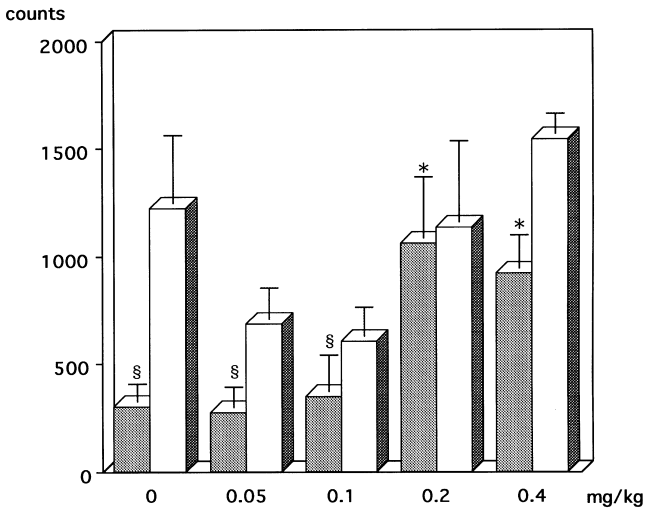

FIG. 2. Effects of acute administration of cabergoline on MPTP-treated or normal control animals. The columns indicate total locomotor activity for 320 min after injection (mean ± SEM). Data in the closed columns are for MPTP-treated animals ($n = 7$), those in the open columns are for normal control animals ($n = 4$). *$p < 0.05$ vs. MPTP-treated animals (0 mg/kg), Kruskal–Wallis test followed by Wilcoxon matched-pairs signed-ranks test §$p < 0.05$ vs. normal control animals (0 mg/kg), Kruskal–Wallis test followed by Mann–Whitney U-test.

kg parkinsonism-like behaviors began to decrease in animals approximately 40 min following injection, and this level of improvement was maintaned until the end of the observation period. The locomotor activities that occurred following treatment with cabergoline were well organized, and none of the animals showed hyperexcitability such as hyperlocomotion or bumping against the cage during treatment. Treated animals did not develop dyskinesias, such as protruding of the tongue, inappropriate opening of the mouth, or chorea of the limbs. Vomiting was not observed at doses of 0.05 or 0.1 mg/kg, but at doses of 0.2 and 0.4 mg/kg, animals did vomit, after which they began to exhibit checking behavior and to increase their locomotor activity.

Effects of Chronic Administration of Cabergoline

Daily administration of cabergoline continuously reversed the akinesia of MPTP-treated animals. The locomotor activity reached its peak on the third day of 22 consecutive days of administration, then it gradually decreased. Normal control animals did not show increased locomotor activity at the dose given, and there were no differences between the locomotor activity of MPTP-treated and normal control animals under chronic treatment with 0.2 mg/kg cabergoline (Fig. 3). After withdrwal of treratment for 7 days, locomotor activity of MPTP-treated animals returned to the level of the pretreatment period. Readministration of cabergoline after a 3-week withdrawal served to reverse the akinesia of MPTP-treated animals as effectively as it did on the first day of chronic administration. Akinesia scores decreased during the chronic administration of cabergoline and also reached their nadir on the third day of the 22-day treatment period. Scores remained low and consistent throughout chronic treatment (Fig. 4). Seven days after the end of chronic cabergoline administration, akinesia scores of MPTP-treated animals returned to those of the pretreatment period. A single readministration of cabergoline 3 weeks after chronic administration again significantly improved the movement of MPTP-treated animals. Neither MPTP-treated nor normal control animals exhibited dyskinesias, such as protrusion of the tongue or inappropriate opening of the mouth, or chorea/choreoathetosis/dystonia of the limbs on chronic administration.

DISCUSSION

Cabergoline has been shown to have potent, selective, and long-lasting dopamine receptor agonistic activity as well as a potent prolactin-lowering effect (1,4,20). In previous treatment of the parkinsonism model used in the current experiment, L-DOPA began to show antiparkinsonian effects a few minutes after administration (7,8), had its peak effect after 60 min, after which it declined. In the current study, single acute administrations of cabergoline reversed the parkinsonism-like symtoms of MPTP-treated common marmosets. The latency to onset of these antiparkinsonian effects was about 60 min af-

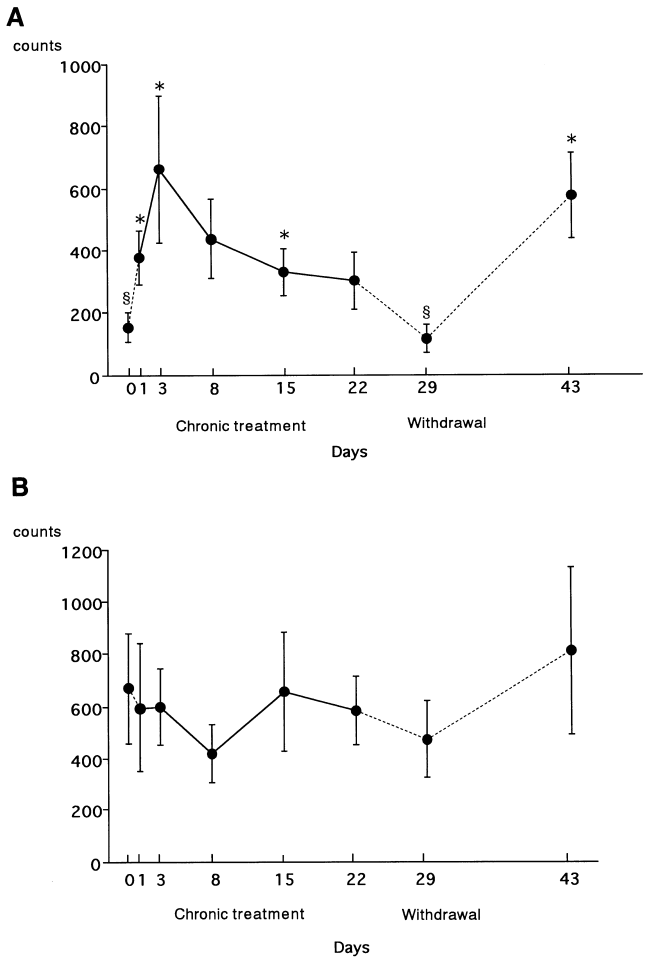

FIG. 3. Effects of chronic administration of cabergoline on locomotor activity of common marmosets. Values indicate mean locomotor activity of animals (mean ± SEM). (A) MPTP-treated animals (n = 7); (B) normal control animals (n = 4). Three weeks after the last daily administration (on day 43), animals received an actute treatment of cabergoline. §$p < 0.05$ vs. normal control animals (day 0), Mann–Whitney U-test *$p < 0.05$ vs. MPTP-treated animals (day 0), Friedman test followed by Wilcoxon matched-pairs signed-ranks test.

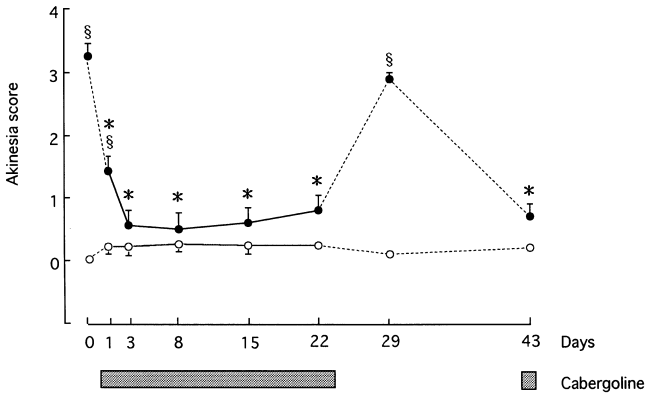

FIG. 4. Effects of chronic administration of cabergoline on akinesia scores of common marmosets. Closed circles indicate mean akinesia scores of MPTP-treated animals (n = 7), open circles indicate those of normal control animals (n = 4). Three weeks after the last daily administration (on day 43), animals received an actute treatment of cabergoline. §$p < 0.05$ vs. normal control animals (day 0), Mann–Whitney test *$p < 0.05$ vs. MPTP-treated animals (day 0), Friedman test followed by Wilcoxon matched-pairs signed-ranks test.

ter injection, which continued throughout the 320 recording period. A dose of 0.2 mg/kg was the minimal dose needed to reverse the akinesia of MPTP-treated animals; however, the antiparkinsonian effect of this dose was relatively long lasting. The present results suggest that cabergoline has long-lasting dopamine receptor agonistic activity in MPTP-treated common marmosets. Although L-DOPA greatly improves the symptoms of Parkinson's disease through its dramatic reversal of akinesia, in just a few years more than 50% of the patients treated with L-DOPA begin to suffer from a wearing-off or short duration of the drug's effect, an instability of motor responses, or L-DOPA-induced dyskinesias (12,18). Several mechanisms are capable of contributing to this short-lived and unpredictable response to L-DOPA. The half-life of L-DOPA is short, and its intestinal absorption and influx to the brain is inhibited by other amino acids (11). These factors appear to have caused the fluctuation of motor disability in L-DOPA treatment (14). Among the dyskinesias induced by antiparkinsonian drugs, diphasic dyskinesias cause severe painful spasms accompanied by profuse sweating, tachycardia, and incapacitating feelings of anxiety and incompetence. These symptoms do not coincide with maximal plasma L-DOPA levels, but rather with levels that are either rising or falling (22). These types of dyskinesias are precipitated by increasing or decreasing stimulation of dopamine receptors, the characteristics of which have been changed by chronic L-DOPA therapy (14). The addition of other dopamine receptor agonists may partially alleviate these responses (3,10). It is hoped that the prolonged antiparkinsonian effects of cabergoline will be helpful in relieving these unplesant side effects of L-DOPA.

The locomotor activity of normal control animals did not increase upon administration of cabergoline: MPTP-treated animals were more sensitive to the locomotor effects of cabergoline. This observation corroborates the finding that a decrease in dopamine neurons induced by MPTP administration caused hypersensitivity in postsynaptic dopamine receptors (15). The locomotor activity of MPTP-treated animals returned to normal control levels at doses of 0.2 mg/kg or 0.4 mg/kg, and animals did not show hyperactivity. Others have showed that high doses of levodopa or apomorphine-induced hyperactivity; activation of postsynaptic dopamine receptors in normal animals increased locomotor activity to a level higher than that seen in control animals (5,21). During chronic cabergoline administration, two of the seven MPTP-treated animals increased their incidence of grooming behavior, but none developed dyskinesias, chorea or any of the other abnormal behaviors that had been observed in MPTP-treated common marmosets during chronic L-DOPA administration (17). The acute effects of L-DOPA on parkinsonism is relatively shorter than that noted here for cabergoline. The longer half-life of cabergoline and its longer effects on dopamine receptors likely explains this finding. Indeed, the additional administration of cabergoline to L-DOPA increased the duration of the "ON" state (the time when drugs reverse parkinsonism) in the treatment of Parkinson's disease (6).

The daily administration of 0.2 mg/kg cabergoline for 22 consecutive days increased locomotor activity in MPTP-treated animals, which peaked on the third day, and then de-

creased gradually. However, akinesia scores remained improved throughout the entire treatment period. The acute administration of cabergoline increased locomotor activity and improved uncoordinated movements of MPTP-treated animals. During repeated daily administration, however, animals showed different responses between the locomotor activity and the improvement of uncoordinated movements. Locomotor activity is mediated by mesolimbic dopaminergic neurons, whereas other behaviors such as stereotypy, are related to activity of the nigrostriatal dopaminergic neurons (9). This difference might explain the inequality between the effects of cabergoline on locomotor activity and on the improvement of uncoordinated movements during daily treatment.

Locomotor activity returned to pretreatment levels in MPTP-treated animals 7 days after the cessation of daily carbegoline administration. Reassessment of cabergoline's effects 3 weeks after the cessation of daily administration showed it was equally efficatious in increasing locomotor activity and reversing other parkinsonism-like symptoms in MPTP-treated animals.

These results suggest that cabergoline, a long-acting dopaminergic agonist, may be a useful agent for the treatment of Parkinson's disease, particulary in patients suffering from fluctuating disabilities.

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