Effect of Cabergoline, a Long-Acting Dopamine D₂ Agonist, on Reserpine-Treated Rodents

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We studied the characterization of cabergoline, a new ergot alkaloid derivative and a selective dopamine D₂ receptor agonist, in comparison to bromocriptine and pergolide in reserpine-treated rodents. Cabergoline (0.25—1.0 mg/kg, s.c.) improved dose-dependently the reserpine-induced akinesia that was assessed on the locomotor activity, and the efficacy lasted longer than those of bromocriptine (1.25—5.0 mg/kg, s.c.) or pergolide (0.0625—0.5 mg/kg, s.c.). Cabergoline (ED₅₀ = 1.10 mg/kg, at 4 h after the administration of drugs) also reversed catalepsy, the failure to correct an externally imposed posture, and its efficacy was stronger and longer than bromocriptine (ED₅₀ = 4.65 mg/kg, at 4 h). Further, reserpine-induced rigidity was improved equally by cabergoline (0.125—1.0 mg/kg, i.v.) and bromocriptine (1.0 mg/kg, i.v.). When cabergoline was administered together with 3-(3,4-dihydroxyphenyl)-l-alanine (l-DOPA), the effects were additive. Our results indicate that the long-lasting effects of cabergoline could be beneficial for treating Parkinson’s disease.

Key words: cabergoline; Parkinson’s disease; akinesia; catalepsy; rigidity

In Parkinson’s disease degeneration of dopaminergic neurons in the substantia nigra causes a decrease in dopaminergic activity. Cabergoline (CG-101, 1-[(6-allylergolin-8β-yl)carbonyl]-1-[(3-dimethylamino)propyl]-3-ethyurea, Fig. 1), a new ergot alkaloid, has higher selectivity and affinity for the dopamine D₂ receptor than for the D₁, D₃, D₅, or 5-HT₃ or 5-HT₂ receptors in vitro. In the present study using reserpine-treated rodents, we examined whether or not cabergoline has any beneficial pharmacological effects on dopamine D₂ receptors in comparison with bromocriptine and pergolide. It is known that animals treated with high doses of reserpine develop catalepsy, akinesia and rigidity, and are thus a laboratory model of parkinsonism. Anti-Parkinson drugs such as l-DOPA, bromocriptine, and pergolide can attenuate the reserpine-induced parkinsonism. Since dopamine receptor agonists have been used clinically in combination with l-DOPA to decrease the dosage and the adverse reactions of l-DOPA, we also studied the combined effect of cabergoline and l-DOPA on reserpine-induced catalepsy in mice.

MATERIALS AND METHODS

Animals: Male ddY mice (Shizuoka Laboratory Animal Center, Japan), weighing 20—35 g, were used for reserpine-induced catalepsy and akinesia. Male Sprague-Dawley rats (Shizuoka Laboratory Animal Center, Japan), weighing 140—160 g, were used for reserpine-induced rigidity. Animals were housed in cages in groups of 5 with free access to commercial food pellets and tap water in a room with a 12-h light/12-h dark cycle (lighting time: 8:00—20:00).

Reagents: Cabergoline (Pharmacia, Milan, Italy) was dissolved in 0.1 mol H₃PO₄ (0.2 ml for each 4.5 mg of cabergoline), and the solution was diluted with saline to the required concentrations (0.12 to 2.0 mg/ml). Bromocriptine (Sigma Chemical Co., St. Louis, MO, U.S.A.) and pergolide (Eli Lilly, Indianapolis, IN, U.S.A.) were dissolved in 0.1 mol tartaric acid and 0.5% (v/v) absolute ethanol, and each solution was diluted with distilled water to the required concentration (bromocriptine: 0.6 to 10 mg/ml, pergolide: 0.06 to 0.5 mg/ml). l-DOPA (concentration: 120 to 200 mg/ml, Sigma Chemical Co., St. Louis, MO, U.S.A.) was dissolved in 6 parts of 0.9% (w/v) NaCl containing 0.1 mol HCl and buffered with 1 part of 7% (w/v) NaHCO₃. Bromocriptine and pergolide doses were expressed in terms of their free base.

Reserpine-Induced Akinesia: Mice were injected intraperitoneally with 5 mg/kg of reserpine (Apoplon Injection, Daiichi Pharmaceutical Co., Ltd., Japan) at 16:00 on the first day of the experiment, and the desired D₂ receptor agonist was injected subcutaneously at 17h.
after the reserpine injection, \textit{i.e.} at 9:00 on the next morning. Locomotor activity was then measured with an Animex 3A device (Shimadzu Seisakusyo, Japan) every hour for 24 h after the agonist injection. Three caged mice were used in these measurements.

**Reserpine-Induced Catalepsy** Mice were injected with reserpine and D$_2$ receptor agonist as described above. Reserpine-induced catalepsy was measured at 1, 2, 4, 6, 8, 10 and 24 h after the administration of the agonist. Evaluation of cataleptic effects was performed according to the procedure of Morelli and Chiara.\textsuperscript{12} Both forepaws of the test mouse were placed on an 8.0-cm high bar. The mouse was considered cataleptic if it remained in this position for 60 s. Five doses of each compound were evaluated, and the ED$_{50}$ value was calculated from the percentage antagonism at each observation time by the Probit method. In the study of cabergoline with L-DOPA, L-DOPA was injected subcutaneously 5 h after the cabergoline or vehicle injection, and the combined effect on the antagonism of catalepsy was measured 30 min after the L-DOPA injection.

**Reserpine-Induced Rigidity** Rigidity in rats was induced by intravenous injection of reserpine at a dose of 10 mg/kg, and the electromyogram (EMG) was monitored before and after the drug injection. Cabergoline was injected intravenously 30 min after the reserpine. The EMG activity was recorded from gastronemius muscle via wire electrodes inserted percutaneously into the muscle after the animals had been placed in Bollman cages and allowed to become calm. The electrical signals were amplified with a bioelectric amplifier (4124, NEC San-ei, Japan) and rectified. The EMG was recorded continuously, and the average integrated activity was determined with an integrator (1322, NEC San-ei, Japan) for 5 min when the foot was stretched at various times. The data on rigidity were expressed as a percent of integrated values before drug administration.

**Statistical Analysis** When the data on akinesia varied among groups by Bartlett test, the data converted to the order. Data were then analyzed by Kruskal–Wallis test followed by Dunnett’s multiple comparison test. Data on rigidity were with appropriate controls by one-way analysis of variance (ANOVA) followed by Dunnett’s multiple comparison test. The ED$_{50}$ and the 95% confidence limits of antagonism of catalepsy were calculated from the percent antagonism of catalepsy by the Probit and Fieller method, respectively. The ED$_{50}$ of cabergoline for catalepsy was compared with that of bromocriptine at the same time by potency ratio test.\textsuperscript{13}

**RESULTS**

Changes in locomotor activity in normal mice through the day were compared with those of reserpine-treated mice (Fig. 2). The activity transiently increased from 13:00 to 14:00 and then remained constant until 20:00 in normal mice, then suddenly increased from 21:00 to 24:00, while the locomotor activity of reserpine-treated mice was much less.

To evaluate the efficacy of the three dopamine agonists, we examined the total locomotor activity over 4 h periods (Fig. 3). Cabergoline attenuated the reserpine-induced akinesia at doses of 0.5 mg/kg or more in a dose-dependent manner, and the effect was continuous over a 24 h period at a dose of 1.0 mg/kg. Bromocriptine improved akinesia at doses of 2.5 and 5.0 mg/kg for 12 h. Pergolide attenuated the akinesia at doses of 0.125 mg/kg or more, and the improvement lasted for 4 h. Pergolide at the dosage used in this experiment had the strongest positive effect on akinesia, whereas cabergoline had the most long-lasting.

The ED$_{50}$ values of cabergoline and bromocriptine on antagonism to reserpine-induced catalepsy at each observation time are shown in Fig. 4. The values of cabergoline were 0.88 to 2.30 mg/kg at 24 h, and those of bromocriptine were 4.46 to 9.15 mg/kg for 10 h; the efficacy of the latter began to decrease at 10 h after the drug administration. When the ED$_{50}$ values of cabergoline and bromocriptine were compared, that of cabergoline was significantly lower at each time period examined.

An intravenous dose of 10 mg/kg of reserpine induced tonic muscular rigidity of the gastrocnemius muscle of rats, but this rigidity disappeared by 80 h after drug administration. Cabergoline improved dose-dependently the muscle condition from 0.125 mg/kg i.v., and at a dose of 1.0 mg/kg lessened the rigidity to the same extent as bromocriptine (Fig. 5). Bromocriptine at an intravenous dose of 1 mg/kg immediately reduced the reserpine-induced rigidity, and the effect continued up to 40 min after administration.

As shown in Table 1, cabergoline at a subcutaneous dose of 1.3 mg/kg showed a 79.2% reversal of the catalepsy, and L-DOPA at a subcutaneous dose of 200 mg/kg also improved the condition by 66.7%. Doses for 30% amelioration of reserpine-induced catalepsy in mice by L-DOPA and cabergoline were 120 and 0.6 mg/kg, s.c., respectively. At these doses, combination treatment of L-DOPA with cabergoline resulted in 66.7% amelioration of the catalepsy, indicating an additive effect when both were used.

**DISCUSSION**

Cabergoline was developed as a new ergot alkaloid derivative like bromocriptine and pergolide, which have
been characterized as selective dopamine D_2 receptor agonists and have been used clinically for treatment of Parkinson's disease. Moreover, cabergoline was shown to bind selectively to central dopamine D_2 receptors in vivo. Ferrai and colleagues showed that a 0.3 mg dose of cabergoline was capable of reducing serum prolactin levels for periods of up to 120 h. From these findings, cabergoline is recognized as having selective and long-lasting activity toward dopamine D_2 receptor.

Reserpine blocks the uptake of noradrenaline, dopamine
and serotonin in the intraneural storage, thereby depleting the brain of these neurotransmitters.\textsuperscript{15} This depletion causes a parkinsonian syndrome in both animals and man.

It has been reported that the akinesia of mice treated with reserpine is only effectively overcome by stimulating dopamine receptors.\textsuperscript{16} On the other hand, catalepsy has been induced not only by reserpine but also by haloperidol, which is a selective dopamine $D_2$ receptor antagonist. Dopamine $D_2$ receptor agonists improved the reserpine-induced catalepsy, whereas SKF 38393, a specific dopamine $D_1$ receptor agonist, did not.\textsuperscript{17} Muscular rigidity is one of the main characteristics of Parkinson’s disease, and it occurs as a side effect of treatment with neuroleptics in human\textsuperscript{17} and animals.\textsuperscript{18} The reserpine-induced rigidity was attenuated by treatment with $\alpha$-DOPA, lisuride and bromocriptine,\textsuperscript{19–21} but since the rigidity did not continue very long it was impossible to quantitatively compare anti-Parkinson drugs. These results support the hypothesis that the dysfunction of dopamine $D_2$ receptors is closely related to the induction of akinesia, catalepsy and rigidity. In this study, we demonstrated the stronger and longer-lasting efficacy of cabergoline toward reserpine-induced akinesia and catalepsy in mice compared with the effects of bromocriptine and pergolide.

It was reported that cabergoline dose-dependently inhibited the specific binding of $[^3H]N$-n-propyl norapomorphine to dopamine receptors in the striatum for 8 h.\textsuperscript{22} Thus, cabergoline does cross the blood-brain barrier and occupies the dopamine receptor for a considerable time. Regarding the qualitative differences between drugs, on the other hand, we reported that cabergoline induced fewer adverse reactions such as hyperactivity at pharmacological doses in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned parkinsonian cynomolgus monkeys than did bromocriptine and pergolide.\textsuperscript{23} $\alpha$-DOPA has been viewed as the most effective drug in the treatment of Parkinson’s disease. In the course of long-term therapy with $\alpha$-DOPA, however, many patients manifest motor fluctuations, psychosis and dyskinesia. In a long-term study, cabergoline ameliorated the motor state fluctuations and decreased the percentage of “off” hours in the first year, and its effect remained constant even later.\textsuperscript{24} Therefore, cabergoline has a strong and long-lasting effect via $D_2$ receptor stimulation with few adverse reactions.

These long-lasting effects could be beneficial for Parkinson’s disease therapy, because the drug’s concomitant use would allow the doses and times of $\alpha$-DOPA administration to be decreased without a reduction in therapeutic effect. This study confirmed the positive combination effect of cabergoline and $\alpha$-DOPA by the experiment on reserpine-induced catalepsy. Rabey et al.\textsuperscript{25} found an influence of bromocriptine on the $\alpha$-DOPA pharmacokinetics in Parkinsonian patients; however, cabergoline reportedly did not influence the $\alpha$-DOPA pharmacokinetic parameters in combination therapy.\textsuperscript{25} Combination treatment of $\alpha$-DOPA with cabergoline may therefore prove highly effective in Parkinson patients.

These findings suggest that the long-lasting effects of cabergoline on dopamine $D_2$ receptors can be beneficial for clinical use since they lessen the fluctuation of effects and adverse events such as dyskinesia seen in $\alpha$-DOPA therapy. Additionally, it is possible to sustain low doses of $\alpha$-DOPA for a long time by combining it with cabergoline. In conclusion, cabergoline produces long-acting dopamine $D_2$ receptor-mediated effects on akinesia and catalepsy in reserpine-treated mice and is also additive with $\alpha$-DOPA in alleviating catalepsy in these mice.

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REFERENCES


