Dopamine Receptor Affinities *in Vitro* and Stereotypic Activities *in Vivo* of Cabergoline in Rats

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An ergot alkaloid derivative, cabergoline, and its metabolites were investigated for their affinities for dopamine D_1 and D_2 receptors in rat striatum *in vitro* in comparison with those of bromocriptine and pergolide. The affinity for D_1 receptors was in the following order: pergolide > des-dimethylaminopropyl cabergoline (FCE21904) > cabergoline $\geq$ bromocriptine $\geq$ des-methyl cabergoline (FCE27395) $\geq$ des-ethylcarbamoyl cabergoline (FCE21590). From the effects of GTP on these affinities for the D_1 receptor, cabergoline, some of its metabolites, and pergolide were characterized as agonists in contrast to bromocriptine which was classified as an antagonist. The affinity for D_2 receptors was ranked as follows: pergolide $\geq$ cabergoline $\geq$ FCE27395 $\geq$ FCE21904 > bromocriptine > FCE21590 > carboxylic acid-type derivative of cabergoline (FCE21589). The affinity of each compound for the D_2 receptor was much higher than that for the D_1 receptor. The selectivity of cabergoline for the D_2 receptor was higher than those of bromocriptine and pergolide. Furthermore, these ergot alkaloids were investigated for eliciting stereotypy after subcutaneous administration to normal rats. Pergolide potently induced stereotypy at doses of 0.5 and 1.0 mg/kg, cabergoline slightly induced it only at a high dose of 2.0 mg/kg, whereas bromocriptine did not induce it at any of the doses tested, 10–40 mg/kg. These results suggest that pharmacological properties of cabergoline for the D_1 and D_2 receptors differ from those of bromocriptine and pergolide.

Key words cabergoline; metabolite; dopamine D_2 -agonist; dopamine D_1 -agonist; stereotypy

Cabergoline (CG-101), 1-[(6-allylergolin-8 β -yl)carbonyl]-1-[3-(dimethylamino)propyl]-3-ethylurea (Fig. 1), is an ergot alkaloid derivative. It was shown to be a potent and selective agonist of dopamine D_2 receptors *in vivo*¹⁾ and to have a long-lasting inhibitory effect on prolactin secretion in rats.²⁾ In hyperprolactinemic patients,³⁾ cabergoline reduced serum prolactin levels. In addition to these anti-prolactin activities, cabergoline improved the Parkinson symptoms in MPTP-treated monkeys,⁴⁾ and clinical treatment with cabergoline for Parkinson's disease was evaluated.^{5,6)}

The disposition and urinary metabolism of 14 C-labeled cabergoline was assessed in rats, monkeys, and humans.⁷⁾ As metabolites in monkey and human urine, FCE21904, FCE21590, FCE21589, and FCE27391 (Fig. 1) were identified by HPLC in comparison with synthetic reference compounds followed by radioactivity detection. Furthermore, FCE27395 (Fig. 1) as a metabolite in rat liver was identified by liquid chromatography-mass spectrometry.⁸⁾ The affinity of these metabolites for D_1 and D_2 receptors *in vitro* seems to be useful for the evaluation of pharmacological properties of cabergoline *in vivo*. However, they have not yet been reported.

In the present study, we investigated and discussed the affinity of cabergoline and its metabolites for D_1 and D_2 receptors *in vitro* and their effect on stereotypy *in vivo* in comparison with those of bromocriptine and pergolide.

MATERIALS AND METHODS

Chemicals Cabergoline, its metabolites (FCE27395, FCE21904, FCE21590, FCE21589, and FCE27391), and pergolide were generously donated by Pharmacia (Milan, Italy). The following compounds were purchased from commercial sources: bromocriptine and sulpiride (Sigma

Chemical Co., St. Louis, MO, U.S.A.), SCH23390 and SKF82958 (Research Biochemicals Inc., Natick, MA, U.S.A.), and [3 H]SCH23390 (71.1 Ci/mmol) and [3 H]-spiperone (18.5 Ci/mmol) (New England Nuclear Research Products, Boston, MA, U.S.A.). Other chemicals were of analytical grade.

Animals Male Sprague-Dawley rats (Japan SLC Inc., Shizuoka, Japan) 7–11 weeks of age were used for binding assays and observation of stereotypy. The animals were housed in groups of 5 per cage with free access to commercial food pellets and tap water and in a room with a 12-h light/12-h dark cycle.

Tissue Preparation for Receptor Binding Assays The

Fig. 1. Chemical Structures of Cabergoline and Its Metabolites

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rats were killed by decapitation, the striatal tissue was dissected out and kept at -80°C until used. The tissue was gently homogenized with a glass-Teflon homogenizer in 100 volumes (100 ml/g wet weight of tissue) of 50 mM Tris-HCl buffer (pH 7.4) for D_1 and D_2 receptor binding assays. The homogenate was centrifuged at $30000 \times g$ for 10 min at 4°C . The obtained pellet was then homogenized in the same way as in the first step. The homogenization and centrifugation steps were repeated a total of three times. The final pellet was resuspended in 25 volumes of 50 mM Tris-HCl buffer (pH 7.4) and kept at -40°C until assayed.

Receptor Binding Assays [^3H]SCH23390 binding (to D_1 receptors) was examined by reference to the methods described by Burt *et al.*⁹⁾ and Andersen and Jansen.¹⁰⁾ A mixture of tissue suspension (2-fold dilution of the above tissue suspension), [^3H]SCH23390 solution (final concentration of 0.5 nM), test compound solution, and Tris-HCl buffer (final concentration of 50 mM, pH 7.4, containing final concentrations of 120 mM NaCl, 5 mM KCl, 2 mM CaCl_2 , and 1 mM MgCl_2) was incubated for 60 min at 30°C and then filtered through a Unifilter plate GFB 96 (Packard Instrument Co., Meriden, CT, U.S.A.) under vacuum. The filter was subsequently washed six times with water and dried. After the addition of 50 μl of scintillation fluid (Micro Sint-0, Packard), the radioactivity was determined with a microplate scintillation counter (Top Count, Packard). Nonspecific binding was determined in the presence of 10 μM SCH23390, and specific binding was estimated by the difference between total and nonspecific binding. In the experiments examining the effect of GTP,¹⁰⁾ the binding assay was performed in the presence of 100 μM GTP. Protein content of the tissue suspension was determined by a bicinchoninic acid protein assay (Pierce, Rockford, IL, U.S.A.).

[^3H]Spiperone binding (to D_2 receptors) was examined with reference to the methods described by Andersen and Jansen,¹⁰⁾ and Creese *et al.*¹¹⁾ A mixture of tissue suspension (2-fold dilution of the frozen tissue suspension), [^3H]spiperone solution (final concentration of 0.2 nM), test compound solution, and Tris-HCl buffer (final concentration of 50 mM, pH 7.4, containing final concentrations of 120 mM NaCl, 5 mM KCl, 1 mM CaCl_2 , and 1 mM MgCl_2) was incubated for 20 min at 37°C followed by vacuum filtration through a Unifilter plate GFB 24 (Packard). The filter was subsequently washed four times with 50 mM Tris-HCl buffer (pH 7.4) and dried. The radioactivity and protein content were determined as above. Nonspecific binding was determined in the presence of 10 μM sulpiride.

Stereotypy Observation of stereotypy was carried out with reference to the method reported by Costall *et al.*¹²⁾ Rats were placed in individual cages (width: 12 cm, height: 15 cm and depth: 21 cm) for 30 min before drug treatment to allow adaptation to their new environment. All observations were made between 12:00 and 16:00 h. Specific stereotypy consisting of sniffing, licking, and biting was monitored. The intensity of the stereotypy was determined from 2-min observation periods at 15, 30, 60, and 120 min after a single subcutaneous administration of each drug to normal rats and was assessed by the scoring system described in Table 1. The sum of the scores at 15,

Fig. 2. Inhibition Curves of Specific [^3H]SCH23390 Binding to Dopamine D_1 Receptors in Rat Striatum by Cabergoline, Bromocriptine, and Pergolide

Cabergoline (●), cabergoline + GTP (○), bromocriptine (■), bromocriptine + GTP (□), pergolide (▲), pergolide + GTP (△). Each point represents the mean $\pm$ S.E. of 3–5 separate experiments.

Table 1. Scoring System Used for Estimation of the Intensity of Stereotypy

Score	Description of stereotypy
0	None
1	Discontinuous sniffing
2	Continuous sniffing or discontinuous licking
3	Continuous licking or discontinuous biting
4	Continuous biting

30, 60 and 120 min after the administration was used to compare the stereotypy achieved by each drug.

Analysis of Data Scatchard plots were analyzed by linear least squares regression analysis. The values of inhibition constant (K_i) were determined by computer fitting with GraphPad PRISMTM software package (GraphPad Software Inc., San Diego, CA, U.S.A.). Stereotypy data were compared with the appropriate control group by Dunnett's multiple comparison test.

RESULTS

Affinity for the D_1 Receptor Specific [^3H]SCH23390 binding in both the absence and presence of 100 μM GTP nearly reached equilibrium after 60 min. Scatchard analysis in the absence of GTP indicated the presence of only one component of the specific [^3H]SCH23390 binding with a dissociation constant (K_d) of 0.291 nM and a maximal number of binding sites (B_{max}) of 0.688 nmol/g protein. These values agreed very closely with the corresponding values (K_d : 0.294 nM, B_{max} : 0.683 nmol/g protein) in the presence of GTP. Figure 2 shows inhibition curves of specific [^3H]SCH23390 binding by cabergoline, bromocriptine, and pergolide. Each compound inhibited the radioligand binding in a concentration-dependent manner. The inhibition by both cabergoline and pergolide was reduced in the presence of 100 μM GTP, while that by bromocriptine was unaffected in the presence of GTP. The inhibition constants (K_i) of cabergoline, its metabolites, bromocriptine, and pergolide for the specific [^3H]SCH23390 binding (to D_1 receptors) are listed in Table 2. Of the ergot alkaloids, pergolide was the most efficient inhibitor of the binding. The affinity of cabergoline

was similar to that of bromocriptine. Based on the affinities of cabergoline and its metabolites for the binding, the inhibitory potency was ranked as follows: FCE21904 > cabergoline ≥ FCE27395 ≥ FCE21590. Neither FCE21589 nor FCE27391 inhibited the binding.

Affinity for the D₂ Receptor Binding of 0.2 nM [³H]-spiperone nearly reached equilibrium after 20 min. [³H]-Spiperone bound with a K_d value of 0.0783 nM and with a B_{max} value of 0.882 nmol/g protein. The inhibition curves

Table 2. Affinities of Cabergoline, Its Metabolites, Bromocriptine, and Pergolide for Dopamine D₁ Receptors in Rat Striatum

	Without GTP		With GTP
	K _i ^H (nM)	K _i ^L (nM)	K _i (nM)
Bromocriptine	2433	—	2926
Pergolide	111	1159	2104
Cabergoline	1724	—	6935
FCE27395	3220	—	7302
FCE21904	474	8370	9805
FCE21590	4194	—	16289
FCE21589	> 30000	—	—
FCE27391	> 30000	—	—
SKF82958	2.61	—	8.12
SCH23390	0.17	—	0.16

Each value was determined by computer fitting from 3–5 separate experiments. K_i^H and K_i^L were inhibition constants (K_i) for high and low affinity binding site, respectively.

Fig. 3. Inhibition Curves of Specific [³H]Spiperone Binding to Dopamine D₂ Receptors in Rat Striatum by Cabergoline (●), Bromocriptine (■), and Pergolide (▲)

Each point represents the mean ± S.E. of 3–5 separate experiments.

of cabergoline, bromocriptine, and pergolide for the [³H]spiperone binding are shown in Fig. 3. These ergot derivatives inhibited the radioligand binding in a concentration-dependent manner. The affinity of ergot alkaloids to the D₂ receptor was ranked in the following order: pergolide ≥ cabergoline > bromocriptine (Table 3). With respect to the affinities of cabergoline and its metabolites (Table 3), their potency was ranked as follows: cabergoline ≥ FCE27395 ≥ FCE21904 > FCE21590 > FCE21589. FCE27391 did not inhibit the binding.

Stereotypy Stereotypy elicited by cabergoline, bromocriptine, pergolide, and SKF82958 after a single subcutaneous administration to normal rats is shown in Fig. 4. SKF82958, a D₁ receptor-selective agonist, clearly induced stereotypy in a dose-dependent manner over the dose range from 0.03 to 0.3 mg/kg. Cabergoline did not significantly induce stereotypy at any dose from 0.5 to 2.0 mg/kg, whereas it slightly induced it at a high dose of 2.0 mg/kg. Pergolide, in contrast, significantly induced stereotypy at doses of 0.5 and 1.0 mg/kg. Bromocriptine was ineffective at all doses from 10 to 40 mg/kg. Furthermore, SCH23390 suppressed the pergolide-induced stereotypy at doses of 0.03 and 0.3 mg/kg.

DISCUSSION

Cabergoline is known to be a potent and selective agonist of D₂ receptors *in vivo*,¹⁾ however, its affinity for D₁ receptors *in vitro* has not been reported. Also, the

Table 3. Affinities of Cabergoline, Its Metabolites, Bromocriptine, and Pergolide for Dopamine D₂ Receptors in Rat Striatum

	K _i ^H (nM)	K _i ^L (nM)
Bromocriptine	6.09	—
Pergolide	0.495	12.4
Cabergoline	0.912	—
FCE27395	1.78	—
FCE21904	2.22	39.1
FCE21590	13.4	—
FCE21589	295	—
FCE27391	> 2500	—

Each value was determined by computer fitting from 3–5 separate experiments. K_i^H and K_i^L were inhibition constants (K_i) for high and low affinity binding site, respectively.

Fig. 4. Stereotypy Elicited by Cabergoline, Bromocriptine, Pergolide, and SKF82958 after a Single Subcutaneous Administration to Normal Rats

Each column represents the mean ± S.E. of 6–8 rats. * and **, significantly different from the control group at p < 0.05 and p < 0.01, respectively.

affinities of its metabolites for D_1 and D_2 receptors *in vitro* have not been reported. Therefore, we investigated the affinities of cabergoline and its metabolites for D_1 and D_2 receptors *in vitro* in comparison with those of bromocriptine and pergolide. The affinity of each compound for the D_2 receptor was much higher than that for the D_1 receptor. The selectivity of cabergoline for the D_2 receptor was higher than those of bromocriptine and pergolide. In addition, the affinities of cabergoline, some of its metabolites, and pergolide for the D_1 receptor were reduced in the presence of GTP; the affinity of bromocriptine was unaffected by GTP. These results suggest that cabergoline, some of its metabolites, and pergolide have agonist-like action toward the D_1 receptor, whereas bromocriptine has antagonist-like action. As regards the affinities of cabergoline and its metabolites for D_1 and D_2 receptors, the affinity of FCE21904 for the D_1 receptor was higher than those of cabergoline and other metabolites, while that of cabergoline for the D_2 receptor was higher than those of its metabolites.

Stereotypy appears after administration of high doses of mixed D_1/D_2 receptor agonists such as apomorphine^{13,14} and ergot derivatives.¹⁵ The present study suggested that the affinities of cabergoline for the D_1 and D_2 receptors *in vitro* are different from those of bromocriptine and pergolide. Therefore, cabergoline was compared with bromocriptine and pergolide in its elicitation of stereotypy *in vivo*. Pergolide potently induced stereotypy, which was inhibited by SCH23390, a D_1 receptor-selective antagonist¹⁶; cabergoline slightly induced it only at a high dose, while bromocriptine did not induce it at any dose tested. These results reflect the potency of these ergot derivatives for the D_1 receptor *in vitro*. Pergolide was found to have agonist-like property toward the D_1 receptor *in vivo* at doses effective in several Parkinson's models.¹⁵ Cabergoline and some of its metabolites also had agonist-like property toward the D_1 receptor *in vitro*, but cabergoline and/or some of its metabolites did not markedly induce stereotypy *in vivo*.

Bromocriptine had an antagonist-like property for the D_1 receptor *in vitro*, but did not induce stereotypy. Therefore, the pharmacological properties of cabergoline with respect to D_1 and D_2 receptors seems to be different from those of bromocriptine and pergolide.

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