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 D₁ and D₂ receptors in rat striatum in vitro in comparison with those of bromocriptine and pergolide. The affinity for D₁ receptors was in the following order: pergolide > des-dimethylaminopropyl cabergoline (FCE21904) > cabergoline ≥ bromocriptine ≥ des-methyl cabergoline (FCE27395) ≥ des-ethylcarbamoyl cabergoline (FCE21590). From the effects of GTP on these affinities for the D₁ 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 D₂ receptors was ranked as follows: pergolide ≥ cabergoline ≥ FCE27395 ≥ FCE21904 > bromocriptine > FCE21590 > carboxylic acid-type derivative of cabergoline (FCE21589). The affinity of each compound for the D₂ receptor was much higher than that for the D₁ receptor. The selectivity of cabergoline for the D₂ 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 D₁ and D₂ receptors differ from those of bromocriptine and pergolide.

Key words cabergoline; metabolite; dopamine D₂-agonist; dopamine D₁-agonist; stereotypy

Cabergoline (CG-101), 1-[(6-allyl-8β-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 D₂ receptors in vivo and to have a long-lasting inhibitory effect on prolactin secretion in rats. In hyperprolactinemic patients, cabergoline reduced serum prolactin levels. In addition to these anti-prolactin activities, cabergoline improved the Parkinson symptoms in MPTP-treated monkeys, and clinical treatment with cabergoline for Parkinson's disease was evaluated. The disposition and urinary metabolism of 14C-labeled cabergoline was assessed in rats, monkeys, and humans. 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. The affinity of these metabolites for D₁ and D₂ 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 D₁ and D₂ 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 [3H]SCH23390 (71.1 Ci/mmol) and [3H]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

\[ \text{Chemical Structures of Cabergoline and Its Metabolites} \]

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 D₁ and D₂ receptor binding assays. The homogenate was centrifuged at 30000 × 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 D₁ receptors) was examined by reference to the methods described by Burt et al., and Andersen and Jansen. 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 CaCl₂, and 1 mM MgCl₂) was incubated for 60 min at 30 °C and then filtered through a Unifilter plate GF/B (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, the binding assay was performed in the presence of 100 μM GTP. Protein content of the tissue suspension was determined by a biocinchoninic acid protein assay (Pierce, Rockford, IL, U.S.A.).

[³H]Spiperone binding (to D₂ receptors) was examined with reference to the methods described by Andersen and Jansen, and Creese et al. 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 CaCl₂, and 1 mM MgCl₂) was incubated for 20 min at 37 °C followed by vacuum filtration through a Unifilter plate GF/B (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.

**Stereotopy** Observation of stereotopy was carried out with reference to the method reported by Costall et al. 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 stereotopy consisting of sniffing, licking, and biting was monitored. The intensity of the stereotopy 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, 30, 60 and 120 min after the administration was used to compare the stereotopy achieved by each drug.

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

**RESULTS**

**Affinity for the D₁ 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 (Kd) of 0.291 nM and a maximal number of binding sites (Bmax) of 0.688 nM/g protein. These values agreed very closely with the corresponding values (Kd: 0.294 nM, Bmax: 0.683 nM/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 (Ki) of cabergoline, its metabolites, bromocriptine, and pergolide for the specific [³H]SCH23390 binding (to D₁ 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]-spermine nearly reached equilibrium after 20 min. [3H]-Spermine 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 of cabergoline, bromocriptine, and pergolide for the [3H]spermine 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.

**Stereotopy** Stereotopy 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 stereotopy in a dose-dependent manner over the dose range from 0.03 to 0.3 mg/kg. Cabergoline did not significantly induce stereotopy 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 stereotopy 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 stereotopy 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, however, its affinity for D1 receptors in vitro has not been reported. Also, the

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

<table>
<thead>
<tr>
<th></th>
<th>Without GTP</th>
<th>With GTP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$K_i^H$ (nm)</td>
<td>$K_i^I$ (nm)</td>
</tr>
<tr>
<td>Bromocriptine</td>
<td>2433</td>
<td>—</td>
</tr>
<tr>
<td>Pergolide</td>
<td>111</td>
<td>1159</td>
</tr>
<tr>
<td>Cabergoline</td>
<td>1724</td>
<td>—</td>
</tr>
<tr>
<td>FCE27395</td>
<td>3220</td>
<td>—</td>
</tr>
<tr>
<td>FCE21904</td>
<td>474</td>
<td>8370</td>
</tr>
<tr>
<td>FCE21590</td>
<td>4194</td>
<td>—</td>
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<td>FCE21589</td>
<td>&gt; 30000</td>
<td>—</td>
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<tr>
<td>FCE27391</td>
<td>&gt; 30000</td>
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<tr>
<td>SKF82958</td>
<td>2.61</td>
<td>—</td>
</tr>
<tr>
<td>SCH23390</td>
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<td>—</td>
</tr>
</tbody>
</table>

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

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**Fig. 3. Inhibition Curves of Specific [3H]Spermine 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.

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

<table>
<thead>
<tr>
<th></th>
<th>$K_i^H$ (nm)</th>
<th>$K_i^I$ (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pergolide</td>
<td>0.495</td>
<td>12.4</td>
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<tr>
<td>Cabergoline</td>
<td>0.912</td>
<td>—</td>
</tr>
<tr>
<td>FCE27395</td>
<td>1.78</td>
<td>—</td>
</tr>
<tr>
<td>FCE21904</td>
<td>2.22</td>
<td>39.1</td>
</tr>
<tr>
<td>FCE21590</td>
<td>13.4</td>
<td>—</td>
</tr>
<tr>
<td>FCE21589</td>
<td>295</td>
<td>—</td>
</tr>
<tr>
<td>FCE27391</td>
<td>&gt; 2500</td>
<td>—</td>
</tr>
</tbody>
</table>

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

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**Fig. 4. Stereotopy 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 D₁ and D₂ receptors in vitro have not been reported. Therefore, we investigated the affinities of cabergoline and its metabolites for D₁ and D₂ receptors in vitro in comparison with those of bromocriptine and pergolide. The affinity of each compound for the D₂ receptor was much higher than that for the D₁ receptor. The selectivity of cabergoline for the D₂ receptor was higher than those of bromocriptine and pergolide. In addition, the affinities of cabergoline, some of its metabolites, and pergolide for the D₁ 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 D₁ receptor, whereas bromocriptine has antagonist-like action. As regards the affinities of cabergoline and its metabolites for D₁ and D₂ receptors, the affinity of FCE21904 for the D₁ receptor was higher than those of cabergoline and other metabolites, while that of cabergoline for the D₂ receptor was higher than those of its metabolites.

Stereotypy appears after administration of high doses of mixed D₁/D₂ receptor agonists such as apomorphine and ergot derivatives. The present study suggested that the affinities of cabergoline for the D₁ and D₂ 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 D₁ receptor-selective antagonist; 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 D₁ receptor in vitro. Pergolide was found to have agonist-like property toward the D₁ receptor in vivo at doses effective in several Parkinson's models. Cabergoline and some of its metabolites also had agonist-like property toward the D₁ 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 D₁ receptor in vitro, but did not induce stereotypy. Therefore, the pharmacological properties of cabergoline with respect to D₁ and D₂ receptors seems to be different from those of bromocriptine and pergolide.

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REFERENCES