Ergoline Derivative LEK-8829-Induced Turning Behavior in Rats with Unilateral Striatal Ibotenic Acid Lesions: Interaction with Bromocriptine

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ABSTRACT
LEK-8829 [9,10-didehydro-N-methyl-(2-propynyl)-6-methyl-8-aminomethylerylergoline bimaleinate] is an antagonist of dopamine D2 receptors and serotonin (5-HT)2 and 5-HT1A receptors in intact animals and a D1 receptor agonist in dopamine-depleted animals. In the present study, we used rats with unilateral striatal lesions with ibotenic acid (IA) to investigate the dopamine receptor activities of LEK-8829 in a model with innervated dopamine receptors. The IA-lesioned rats circled ipsilaterally when challenged with apomorphine, the mixed agonist on D2 receptors and serotonin (5-HT)2 and 5-HT1A receptors and was designed as a potential atypical antipsychotic drug (Krisch et al., 1994, 1996). In vitro experiments have shown that LEK-8829 stimulates adenylate cyclase (Panlabs) and binds to dopamine D1 receptors with moderate affinity (Krisch et al., 1994). On the other hand, the drug has been tested in vivo for its effects on dopamine D1 receptors only in dopamine-depleted animals, using the rats with unilateral lesions of striatonigral neurons with 6-hydroxydopamine (6-OHDA). In this turning model, LEK-8829 induced a dose-dependent contralateral turning that was blocked by dopamine D1 receptor antagonist SCH-23390. The treatment with D1 receptor agonist SKF-82958 induced ipsilateral turning, whereas the treatment with D2 receptor antagonist haloperidol induced contralateral posture. The combined treatment with SKF-82958 and haloperidol resulted in a weak contralateral turning, indicating the possible receptor mechanism of contralateral turning induced by LEK-8829. Bromocriptine induced a weak ipsilateral turning that was blocked by haloperidol. The ipsilateral turning induced by bromocriptine was significantly potentiated by the coadministration of a low dose but not by a high dose of LEK-8829. The potentiation of turning was blocked either by SCH-23390 or by haloperidol. The potentiation of ipsilateral turning suggests the costimulation of D2 and D1 receptors by bromocriptine and LEK-8829, respectively, whereas the lack of potentiation by the highest dose of LEK-8829 may be explained by the opposing activity of LEK-8829 and bromocriptine at D2 receptors. We propose that the D2 and 5HT2 receptor-blocking and D1 receptor-stimulating profile of LEK-8829 is promising for the treatment of negative symptoms of schizophrenia.

The ergoline derivative LEK-8829 [9,10-didehydro-N-methyl-(2-propynyl)-6-methyl-8-aminomethyleryl ergoline bimaleinate] is an antagonist on dopamine D2 receptors and on serotonin (5-HT)2 and 5-HT1A receptors and was designed as a potential atypical antipsychotic drug (Krisch et al., 1994, 1996). In vitro experiments have shown that LEK-8829 stimulates adenylate cyclase (Panlabs) and binds to dopamine D1 receptors with moderate affinity (Krisch et al., 1994). On the other hand, the drug has been tested in vivo for its effects on dopamine D1 receptors only in dopamine-depleted animals, using the rats with unilateral lesions of striatonigral neurons with 6-hydroxydopamine (6-OHDA). In this turning model, LEK-8829 induced a dose-dependent contralateral turning and the expression of c-fos mRNA in dopamine-depleted striatum that were both blocked by SCH-23390 [(R)-(+)7-chloro-8-hydroxymethyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride], a dopamine D1 receptor antagonist, but not by haloperidol (dopamine D2 receptor antagonist) or pindolol (5-HT1A receptor antagonist). It was therefore proposed that LEK-8829 may be an agonist on dopamine D1 receptors (Živin et al., 1996). The interaction of LEK-8829 and bromocriptine has also been analyzed in a 6-OHDA model (Živin et al., 1998). It was found that contralateral turning that was initiated with bromocriptine was not inhibited by the treatment with either LEK-8829 or SCH-23390, whereas the combined treatment with both drugs inhibited the turning. It was concluded that LEK-8829 has a dual action on the dopamine receptors in dopamine-depleted striatum, as an agonist on dopamine D1 receptors and as an antagonist on dopamine D2 receptors (Živin et al., 1998).

Drugs with a D2 and 5HT2 receptor-blocking and D1 receptor-stimulating profile are promising for the treatment of the negative symptoms of schizophrenia (Kahn and Davis, 1995; Okubo et al., 1997; Lidow et al., 1998). Because the dopamine depletion does not seem to be involved in the pathogenesis of...
schizophrenia, it is appropriate to study the effects of such drugs in animals with normosensitive dopamine receptors. The rats with unilateral striatal lesion with excitotoxic glutamate analogs (kainic, ibotenic, or quinolinic acid) may be used for this purpose. In the striatum injected with an excitotoxin, a degeneration and death of intrinsic neurons occur, whereas the afferent neurons and fibers on passage are relatively spared (McGeer and McGeer, 1976; Christensen-Nylander et al., 1986). Striatal degeneration on the lesioned side leads to the functional imbalance of the basal ganglia (Fields et al., 1978; Mayer et al., 1990), which results in the turning behavior in response to directly or indirectly acting dopamine agonists. In this model, the effects of dopamine drugs are preferentially mediated by the dopamine-activated dopamine receptors of the intact striatum. The rats with unilateral striatal lesion with excitotoxins respond with the ipsilateral turning behavior after application of mixed dopamine D2/D1 receptor agonists, such as apomorphine (Schwarz et al., 1979; Herrera-Marschitz and Ungerstedt, 1984; Barone et al., 1986; Fricker et al., 1996). Only a low-intensity ipsilateral turning develops after the application of more selective dopamine D2 receptor agonists such as bromocriptine (Schwarz et al., 1979) or LY-171555 [(+)-3-3-(hydroxyphenyl)-N-n-propylpiperidinide] (Barone et al., 1986). The induction of ipsilateral turning by D2 receptor agonist depends on the costimulation of dopamine D1 receptors by endogenous dopamine or by exogenously applied D1 receptor agonists (Dziewczapolski et al., 1997). The concept of requisite synergism of dopamine innervated dopamine receptors (LaHoste and Marczapolski et al., 1997) could be used to explain some aspects of dopamine receptor interactions involved in the mediation of ipsilateral turning in rats with excitotoxic striatal lesion.

The aim of the present study was to investigate the effects of LEK-8829 on normosensitive dopamine receptors. We hypothesized the blockade of dopamine D2 receptors and concomitant stimulation of dopamine D1 receptors by LEK-8829. The rats with unilateral striatal lesions induced with ibotenic acid (IA) were used to resolve this issue. The properties of LEK-8829 were analyzed using SCH-23390, a selective dopamine D1 receptor antagonist; haloperidol, a D2 receptor antagonist; SKF-82958, a selective dopamine D1 receptor agonist; and bromocriptine, a clinically used D2 receptor agonist; and bromocriptine, which was injected i.p.

### IA-Induced Striatal Lesions
The stereotaxic lesions were performed on experimentally naive rats. The animals were anesthetized with the i.p. injection of xylazine (8 mg/kg; Rompun, Bayer, Leverkusen, Germany), ketamine (60 mg/kg; Ketanest, Parke-Davis, Wien, Austria), and atropine (0.6 mg/kg; Belupo, Koprivnica, Croatia) and placed in a stereotaxic frame (Trent Wells, South Gate, CA). IA (RBI) was infused at a rate of 0.15 μl/min at three different injection sites (each site, 0.5 μl of 0.06 M ibotenic acid dissolved in 0.1 M phosphate buffer, pH 7.4) into the right neostriatum at the following coordinates: A = 0.2, L = 3.0, V = 4.0, and V = 5.5 and A = 1.5, L = 2.5, and V = 4.6 (all coordinates are given in millimeters, anterior coordinate from bregma, lateral coordinate from the midline, and ventral coordinate from the surface of the skull at bregma; stereotaxic coordinates according Paxinos and Watson, 1982). The incisor bar was set 2.3 mm below the interaural line. The infusion was delivered via a 30-gauge stainless steel cannula connected by polyethylene tubing to a 10-μl Hamilton syringe mounted on a microdrive pump (Harvard Apparatus, South Natick, MA). At each injection site, the cannula was left in place for 2 min before retraction. After surgery, the lesioned animals were left for 14 days to recover and to allow for neuronal degeneration.

### Recording of Turning Behavior
Each rat was placed in a plastic cylindrical chamber (40-cm diameter) of the Labline automated rotometer system (Colbourn Instruments, Allentown, PA) designed for the simultaneous electromechanical recording (Ungerstedt and Arbuthnott, 1970) of the turning behavior of eight animals. The data files of the turning profiles of each animal (i.e., the full left/right turns per minute) recorded by the L2TS data acquisition software (Colbourn Instruments) were graphically represented and analyzed using standard Lotus 1–2–3 spreadsheet, running on a PC.

### Apomorphine Test
To determine the development of striatal degeneration and to stabilize the turning response, the IA-lesioned animals were primed to the stimulation of dopamine D1 and D2 receptors by the treatment with apomorphine hydrochloride (5 mg/kg) in the third and fourth postoperative weeks. Only the apomorphine-primed IA-lesioned rats responding with at least 300 contralateral turns during the second apomorphine session were used in subsequent experiments. The animals were then randomly divided into experimental groups for experiments with drugs. The experiments with drugs started 1 week after the second priming session with apomorphine.

### Dose-Response of LEK-8829-Induced Contralateral Turning
The dose-response of LEK-8829-induced turning (i.e., the cumulative number of contralateral turns, duration of turning behavior, peak turning frequency, mean turning frequency, and onset of turning) was assessed using five groups of IA-lesioned animals. Each group received one of the following doses of LEK-8829: 0.5, 1, 3, or 15 mg/kg or saline.

#### Effect of SCH-23390 on Contralateral Turning Behavior Induced by LEK-8829
A group of four animals was treated in three experimental sessions as follows: 1) saline + LEK-8829 (3 mg/kg), 2) SCH-23390 (0.5 mg/kg) + LEK-8829 (3 mg/kg), and 3) SCH-23390 (1 mg/kg) + LEK-8829 (3 mg/kg). The saline + SCH-23390 pretreatment was given 20 min before the administration of LEK-8829. Experimental sessions were performed in weekly intervals.

### Effect of Haloperidol on Ipsilateral Turning Behavior Induced by SKF-82958
A group of five animals was treated in four experimental sessions as follows: 1) saline + SKF-82958 (3 mg/kg), 2) haloperidol (0.25 mg/kg) + SKF-82958 (3 mg/kg), 3) haloperidol (0.25 mg/kg) + saline, and 4) saline + saline. The saline + haloperidol pretreatment was given 20 min before the administration of SKF-82958 saline. There was a drug-free interval of 1 week between each experimental session. The cumulative number of contralateral and ipsilateral turns and the maximum turning frequency (peak turning frequency) were registered during a 240-min period. The average...
number of cumulative ipsilateral turns was compared with the average number of cumulative contralateral turns using a two-tailed paired Student’s t test (p < .05) to verify the statistical significance of the direction of turning.

**Dose Dependence of Bromocriptine/LEK-8829 Interaction.** In the first experiment, a group of four animals received injections of bromocriptine at 0 min and saline + LEK-8829 at 120 min in seven experimental sessions as follows: 1) bromocriptine (30 mg/kg) + saline, 2) bromocriptine (30 mg/kg) + LEK-8829 (0.5 mg/kg), 3) bromocriptine (30 mg/kg) + LEK-8829 (1 mg/kg), 4) bromocriptine (30 mg/kg) + LEK-8829 (3 mg/kg), 5) bromocriptine (30 mg/kg) + LEK-8829 (10 mg/kg), 6) bromocriptine (30 mg/kg) + LEK-8829 (30 mg/kg), and 7) bromocriptine (30 mg/kg) + saline. There was a drug-free interval of 1 week between each experiment showing the total number of turns and the duration of turning behavior induced by LEK-8829 is shown in Table 1. With lower doses of LEK-8829 (i.e., 0.5 and 1 mg/kg), the animals adopted contralateral posture, with a few contralateral turns that were observed for 2 h after the injection of the drug. With higher doses of LEK-8829 (3 and 15 mg/kg), the contralateral turning become more vigorous, with significantly increased number of cumulative number of turns, duration of turning behavior, and turning frequency (p < .05; ANOVA followed with Scheffe’s multiple-comparison test).

**Effect of SCH-23390 on Haloperidol on Bromocriptine/LEK-8829 Interaction.** Five groups of four animals received injections of either bromocriptine or saline at 0 min and LEK-8829 at 120 min as follows: 1) saline + LEK-8829 (3 mg/kg), 2) bromocriptine (3 mg/kg) + LEK-8829 (3 mg/kg), 3) bromocriptine (10 mg/kg) + LEK-8829 (3 mg/kg), and 4) bromocriptine (30 mg/kg) + LEK-8829 (3 mg/kg). The cumulative number of turns recorded in 2 h after the injection of LEK-8829 was calculated and used for statistical evaluation.

In the second experiment, four groups of four animals received injections of either bromocriptine or saline at 0 min and LEK-8829 at 120 min as follows: 1) saline + LEK-8829 (3 mg/kg), 2) bromocriptine (3 mg/kg) + LEK-8829 (3 mg/kg), 3) bromocriptine (10 mg/kg) + LEK-8829 (3 mg/kg), and 4) bromocriptine (30 mg/kg) + LEK-8829 (3 mg/kg). The cumulative number of ipsilateral turns recorded in 2 h after the third injection was calculated for each experimental group and used for statistical evaluation.

**Histological Visualization of IA Lesions.** At the completion of behavioral experiment, eight representative animals (apomorphine-induced ipsilateral rotations, 472 ± 94) were decapitated while under CO2 anesthesia, and the brains were rapidly frozen on dry ice and stored at −70°C until further processing. Six evenly spaced striatal cryostat sections (10 μm) were cut between anteriorposterior coordinates + 2.2 mm and −1.3 mm relative to bregma from eight representative animals. The total area of the spared portion on the lesioned side was measured (mm²) and compared with the total striatal area of intact side.

**Statistical Analysis.** All values are expressed as mean ± S.E.M. number of turns during the observation period, where n represents the number of animals. Statistical evaluation of the effect of treatments between experimental sessions performed on the same group of animals and for the statistical evaluation of the effect of treatments between different groups of animals was evaluated using one-way ANOVA followed with Scheffe’s multiple-comparison test. A two-tailed paired Student’s t test was used to verify the statistical significance of the direction of turning in the haloperidol/SKf experiment because the animals did not turn exclusively in one direction.

## Results

**Dose-Response of LEK-8829-Induced Contralateral Turning in IA Model.** The result of the dose-response experiment showing the total number of turns and the duration of turning behavior induced by LEK-8829 is shown in Table 1. With lower doses of LEK-8829 (i.e., 0.5 and 1 mg/kg), the animals adopted contralateral posture, with a few contralateral turns that were observed for 2 h after the injection of the drug. With higher doses of LEK-8829 (3 and 15 mg/kg), the contralateral turning became more vigorous, with significantly increased number of cumulative number of turns, duration of turning behavior, and turning frequency (p < .05; ANOVA followed with Scheffe’s multiple-comparison test).

**Effect of SCH-23390 on Contralateral Turning Behavior Induced by LEK-8829.** The pretreatment with SCH-23390 resulted in a dose-dependent inhibition of LEK-8829-induced contralateral turning. After the pretreatment with SCH-23390 at the dose of 0.5 mg/kg, the total number of contralateral turns induced by LEK-8829 (3 mg/kg) was nonsignificantly reduced, whereas the pretreatment with SCH-23390 with the dose of 1 mg/kg resulted in almost complete inhibition of contralateral turning induced by LEK-8829 (3 mg/kg) by 70%, n = 4, p > .05, and by 90%, n = 4, p < .05, respectively, compared with the cumulative number of contralateral turns recorded in a control, saline-pretest conditions.

### Table 1

Dose-response of turning behavior after injection of LEK-8829

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Dose (mg/kg)</th>
<th>Cumulative Contralateral Turns</th>
<th>Duration of Turning (min)</th>
<th>Peak Turning Frequency (turns/min)</th>
<th>Mean Turning Frequency (turns/min)</th>
<th>Onset Time of Turning (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>4</td>
<td>0.5</td>
<td>19 ± 3</td>
<td>61 ± 5</td>
<td>1.8 ± 0.3</td>
<td>0.3 ± 0.1</td>
<td>7.3 ± 1.5</td>
</tr>
<tr>
<td>B</td>
<td>8</td>
<td>1.0</td>
<td>34 ± 8</td>
<td>134 ± 12</td>
<td>2.3 ± 0.5</td>
<td>0.2 ± 0.0</td>
<td>6.8 ± 1.7</td>
</tr>
<tr>
<td>C</td>
<td>12</td>
<td>3.0</td>
<td>192 ± 29</td>
<td>168 ± 6</td>
<td>3.5 ± 0.5</td>
<td>1.1 ± 0.2</td>
<td>5.3 ± 1</td>
</tr>
<tr>
<td>D</td>
<td>4</td>
<td>15.0</td>
<td>488 ± 136</td>
<td>240 ± 16</td>
<td>6.3 ± 1.5</td>
<td>2.0 ± 0.6</td>
<td>4.0 ± 0.1</td>
</tr>
<tr>
<td>E</td>
<td>4</td>
<td>Saline</td>
<td>2 ± 0.5</td>
<td></td>
<td>1.0 ± 0.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

One-way ANOVA with Scheffe’s multiple-comparison test (p < .05); cumulative contralateral turns: a significantly different from groups C and D; b significantly different from groups A, B, C, and E; c significantly different from groups A, B, and C; D; e significantly different from groups A, B, C, and E; f significantly different from groups C and D. Peak frequency of turning: a significantly different from group E; b significantly different from groups D and E; c significantly different from groups A, B, C, and E; d significantly different from groups C and D. Mean frequency of turning: a significantly different from group D; d significantly different from groups C and D; e significantly different from group B; f significantly different from groups A and B.

Morphometrical analysis of the extent of striatal lesions induced with IA was performed by using the MCID, M4 image analyzer (Imaging Research Inc., Canada). Six different levels between −2.2 mm and −1.3 mm relative to bregma were evaluated from eight representative animals. The total area of the spared portion on the lesioned side was measured (mm²) and compared with the total striatal area of intact side.
potentiation was then replaced by the inhibition of turning, which lasted for about 1 h. The inhibition of turning was followed by a gradual reappearance of ipsilateral turning (Fig. 3B and see Fig. 5C). At about 4 h after the injection of LEK-8829, the animals changed the direction of turning to the contralateral side (Fig. 3B). The cumulative number of turns and the turning profiles recorded in the last bromocriptine/saline session were not significantly different from the control value obtained in the first bromocriptine/saline session (n = 4, p > .05, one-way ANOVA with Scheffe’s multiple-comparison test) (Fig. 2).

The second experiment revealed the effect of different doses of bromocriptine pretreatment on contralateral turning induced by LEK-8829. Pretreatment with saline or bromocriptine at the dose of 3 mg/kg did not significantly affect the contralateral turning induced by 3 mg/kg of LEK-8829 (Fig. 4). Bromocriptine pretreatment at the dose of 3 mg/kg per se induced only a few ipsilateral turns (observed in 2-h period before the injection of LEK-8829; Fig. 5E). On the other hand, the pretreatment with bromocriptine at the dose of 10 or 30 mg/kg induced a significant ipsilateral turning response that was potentiated by the injection of LEK-8829 (3 mg/kg) (Fig. 5, B and F). The cumulative number of ipsilateral turns recorded in 2 h after the injection of LEK-8829 was dependent on the dose of bromocriptine used in the pretreatment (Fig. 4).

Effect of SCH-23390 and Haloperidol on Bromocriptine/LEK-8829 Interaction. The experiment with specific antagonists of dopamine receptors D1 and D2 (1 mg/kg SCH-23390 and 5 mg/kg haloperidol, respectively) has shown that both antagonists almost completely inhibited the ipsilateral turning response mediated by the bromocriptine (30 mg/kg/LEK-8829 (3 mg/kg) combination (by 99% and 93%, respectively, compared with the effect of saline in the control bromocriptine/LEK-8829 group, n = 4, p < .05, one-way ANOVA with Scheffe’s multiple-comparison test). The experiment also revealed a significant inhibition of bromocriptine-induced turning in response to haloperidol (by 90%; n = 4, p < .05, one-way ANOVA with Scheffe’s multiple-comparison test) (Fig. 6).

Visualization of Position and Extent of Striatal Lesions. The AChE histochemistry performed on eight representative animals has shown extensive excitotoxic lesions positioned in the central striatum as shown in Fig. 7. Histo- metre analysis revealed that the IA-injection destroyed 50 to 70% of the striatal tissue (Table 3). This resulted in the shrinkage of the striatum and enlargement of lateral ventricle on the lesioned side. Less intense AChE staining was found in the frontoparietal cortex overlying the lesioned striatum. Other extrastriatal regions were not visibly affected.

**Discussion**

Induction of Contralateral Turning in Response to LEK-8829. In rats with excitotoxic striatal lesions, the dopamine receptor agonists induce ipsilateral turning (Schwarcz et al., 1979; Herrera-Marschitz and Ungerstedt, 1984; Dunnett et al., 1988; Norman et al., 1990). The ipsilateral turning is generally less intense than the contralateral turning induced in rats with unilateral dopaminergic striatal deafferentation with 6-OHDA, which are supersensitive to dopamine receptor agonists (Schwarcz et al., 1979, Herrera-
TABLE 2
Effect of haloperidol on SKF-82958-induced turning behavior
A group of five IA-lesioned rats were treated in weekly intervals as follows: apomorphine (5 mg/kg), saline + SKF-82958 (3 mg/kg), haloperidol (0.25 mg/kg) + SKF-82958 (3 mg/kg), haloperidol (0.25 mg/kg) + saline, and saline + saline. Cumulative number of turns and peak turning frequency were calculated for contralateral and ipsilateral turning after each treatment for 240 min. Data are expressed as mean ± S.E.M. (n = 5).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cumulative number of turns</th>
<th>Peak turning frequency</th>
<th>Cumulative number of turns</th>
<th>Peak turning frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(min)</td>
<td>(turns/min)</td>
<td>(min)</td>
<td>(turns/min)</td>
</tr>
<tr>
<td>Apomorphine</td>
<td>6.1 ± 0.8</td>
<td>1.6 ± 0.8</td>
<td>3.9 ± 3.0</td>
<td>1.5 ± 0.4</td>
</tr>
<tr>
<td>Saline/SKF</td>
<td>12.2 ± 7.7</td>
<td>1.6 ± 0.8</td>
<td>451 ± 80.1</td>
<td>9.6 ± 1.5</td>
</tr>
<tr>
<td>Haloperidol/SKF</td>
<td>47.4 ± 27.7**</td>
<td>1.8 ± 0.4</td>
<td>165.8 ± 50.7*</td>
<td>5.8 ± 1.1</td>
</tr>
<tr>
<td>Haloperidol/saline</td>
<td>4.8 ± 2.4</td>
<td>1.0 ± 0.4</td>
<td>1.2 ± 0.4</td>
<td>0.8 ± 0.3</td>
</tr>
<tr>
<td>Saline/saline</td>
<td>14.4 ± 4.4</td>
<td>1.5 ± 0.3</td>
<td>13.5 ± 2.7</td>
<td>1.8 ± 0.5</td>
</tr>
</tbody>
</table>

*p < .05, paired two-tailed Student’s t test, statistically significant ipsilateral turning.
**p < .05, paired two-tailed Student’s t test, statistically significant contralateral turning.

Fig. 2. Bromocriptine/LEK-8829 interaction in IA-lesioned rats. Columns represent mean cumulative ipsilateral turns recorded during 2 h after administration of saline/LEK-8829; error bars indicate S.E.M. (n = 4). Saline + LEK-8829 treatment was given 120 min after administration of bromocriptine. Experimental sessions were performed at weekly intervals as follows: 1) BRO (30) + SAL (w1) (30 mg/kg bromocriptine, saline, first week), 2) BRO (30) + LEK (0.5) (30 mg/kg bromocriptine, 0.5 mg/kg LEK-8829), 3) BRO (30) + LEK (1) (30 mg/kg bromocriptine, 1 mg/kg LEK-8829), 4) BRO (30) + LEK (3) (30 mg/kg bromocriptine, 3 mg/kg LEK-8829), 5) BRO (30) + LEK (10) (30 mg/kg bromocriptine, 10 mg/kg LEK-8829), 6) BRO (30) + LEK (30) (30 mg/kg bromocriptine, 30 mg/kg LEK-8829), and 7) BRO (30) + SAL (w7) (30 mg/kg bromocriptine, saline, last week). Statistical evaluation: significantly increased total number of ipsilateral turns *compared with groups BRO (30) + SAL (w1), BRO (30) + LEK (30), BRO (30) + SAL (w7), (+) compared with BRO (30) + LEK (0.5), and †compared with BRO (30) + SAL (w1), BRO (30) + LEK (30) (n = 4, p < .05, one-way ANOVA with Scheffe’s multiple-comparison test).

Fig. 3. Individual recordings of turning behavior of representative IA-lesioned rat showing effect of high dose of LEK-8829 on bromocriptine-induced ipsilateral turning. In the first experimental session, the rat received injection of 30 mg/kg bromocriptine [BRO (30)] at 0 min, followed by injection of saline (SAL) at 120 min (A). In the second experimental session, the same rat was treated with 30 mg/kg bromocriptine [BRO (30)] at 0 min, followed by the injection of 30 mg/kg LEK-8829 [LEK (30)] at 120 min (B). Profiles show rotational speed (turns/min) recorded for 10 h. Above zero line, contralateral rotations; below zero line, ipsilateral rotations. Arrows indicate the timing of drug/saline injections. Note initial inhibition of ipsilateral turning induced by LEK-8829 and a change of direction of turning (from ipsilateral to contralateral) 4 h after the treatment with LEK-8829.

Haloperidol and Ungerstedt, 1984). Few reports of turning induced with apomorphine or amphetamine have shown the dependence of the development of striatal degeneration and to prevent further sensitization in subsequent experiments (cf. Norman et al., 1990). When challenged with apomorphine, all the rats used in our experiments turned ipsilaterally.

The induction of dose-dependent contralateral turning in IA-lesioned rats with LEK-8829 was therefore not expected. The LEK-8829-induced turning was blocked by SCH-23390, indicating a mechanism linked to dopamine D1 receptors. To further analyze the receptors that were potentially involved in the induction of contralateral turning, we injected a specific dopamine D1 receptor agonist, SKF-82958, into haloperidol-pretreated rats. In vehicle-pretreated rats, SKF-82958 induced a weak ipsilateral turning, whereas in haloperidol-pretreated rats, SKF-82958 induced a weak contralateral turning. Haloperidol per se induced only a contralateral rotation. It has been described previously that in rats and mice with different types of unilateral striatal lesions that neuroleptics induced a postural asymmetry of the head, neck, and body, away from the side of lesion (for review, see Pycock, 1980).

Striatal dopamine D1 and D2 receptors seem to be located at functionally distinct sites (Herrera-Marschitz and Unger-
ANOVA with Scheffe’s multiple-comparison test). Compared with group BRO (10)
increased total number of ipsilateral turns in group BRO (30) LEK (3) (30 mg/kg bromocriptine, 3 mg/kg LEK-8829).

*Significantly increased total number of ipsilateral turns in group BRO (30) + LEK (3) compared with group BRO (10) + LEK (5) (n = 4, p < .05, one-way ANOVA with Scheffe’s multiple-comparison test).

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Fig. 4. Bromocriptine/LEK-8829 interaction in IA-lesioned rats. Columns represent mean cumulative turns recorded during 2 h after administration of LEK-8829. Error bars indicate S.E.M. (n = 4). IPSI, ipsilateral turning; CONTRA, contralateral turning. Four groups of rats received LEK-8829 injections 120 min after administration of saline/bromocriptine as follows: 1) SAL + LEK (3) (saline, 3 mg/kg LEK-8829), 2) BRO (3) + LEK (3) (3 mg/kg bromocriptine, 3 mg/kg LEK-8829), 3) BRO (10) + LEK (3) (10 mg/kg bromocriptine, 3 mg/kg LEK-8829), and 4) BRO (30) + LEK (3) (30 mg/kg bromocriptine, 3 mg/kg LEK-8829).

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Fig. 5. Turning profiles of bromocriptine/LEK-8829 interaction in IA-lesioned rats. (A–F) Rotational speed (turns/min) of individual rats recorded for 6 h. Rats were injected with bromocriptine/saline at 0 min, followed by injection of LEK-8829/saline at 120 min (arrows) as follows: A, 30 mg/kg bromocriptine [BRO (30)], saline (SAL); B, 30 mg/kg bromocriptine [BRO (30)], 3 mg/kg LEK-8829 [LEK (3)]; C, 30 mg/kg bromocriptine [BRO (30)], 30 mg/kg LEK-8829 [LEK (30)]; D, saline (SAL), 3 mg/kg LEK-8829 [LEK (3)]; E, 3 mg/kg bromocriptine [BRO (3)], 3 mg/kg LEK-8829 [LEK (3)]; and F, 10 mg/kg bromocriptine [BRO (10)], 3 mg/kg LEK-8829 [LEK (3)]. Above zero line, contralateral rotations; below zero line, ipsilateral rotations.

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Fig. 6. Effect of SCH-23390 and haloperidol on turning profile of bromocriptine/LEK-8829 interaction in IA-lesioned rats. Columns represent mean cumulative ipsilateral turns recorded during 2 h after the administration of LEK-8829/saline; error bars indicate S.E.M. (n = 4). Each group was treated with bromocriptine at 0 min, followed by drug/saline injections at 100 and 120 min as follows: BRO + SAL + SAL (30 mg/kg bromocriptine, saline, saline); BRO + SAL + LEK (30 mg/kg bromocriptine, saline, 3 mg/kg LEK-8829); BRO + SCH + LEK (30 mg/kg bromocriptine, 1 mg/kg SCH-23390, 3 mg/kg LEK-8829); BRO + HAL + LEK (30 mg/kg bromocriptine, 5 mg/kg haloperidol, 3 mg/kg LEK-8829); BRO + HAL + SAL (30 mg/kg bromocriptine, 5 mg/kg haloperidol, saline). Statistical evaluation: *significantly decreased total number of turns in groups BRO + SCH + LEK, BRO + HAL + LEK, and BRO + HAL + SAL compared with BRO + SAL + LEK group; †significantly decreased total number of turns in groups BRO + HAL + SAL and BRO + SCH + LEK compared with BRO + SAL + SAL group (for all comparisons: n = 4, p < .05, one-way ANOVA with Scheffe’s multiple-comparison test).

contrast, phasic activation of the indirect pathway leads to increased basal ganglia output and to suppression of movement (Wichmann and DeLong, 1996). The overall effect of dopamine release is to reduce basal ganglia output, leading to increased activity of thalamocortical projection neurons (Gerfen, 1995). It may be implied that D₄ agonists inhibit, whereas D₂ antagonists stimulate, the striatal neurons of the indirect pathway, facilitating and suppressing the movement, respectively. On the other hand, haloperidol does not seem to have such effects on the IA-lesioned side. A partial loss of striatal dopamine D₂ receptors in the IA-lesioned striatum (Mayer et al., 1990) may provide an explanation for the diminished effects of haloperidol on the lesioned side. A preferential degeneration of the D₂ receptor-bearing neurons of the indirect striatonigral pathway that would lead to the disinhibition of thalamocortical neurons has been proposed to explain the mechanism for the development of dyskinesia in Huntington’s chorea (Albin et al., 1992). The excitotoxic lesion induced with IA, however, is not selective in this respect and also affects the D₁ receptor-bearing neurons of the direct striatonigral efferents (Arai et al., 1987; Mansour et al., 1992). On the other hand, a transneuronal degeneration of substantia nigra reticulata may develop after the striatal lesion with IA (Saji et al., 1997; DeGiorgio et al., 1998). The transneuronal degeneration of the GABAergic nigrothalamic neurons of the pars reticulata therefore seems to be a more likely cause for the disinhibition of thalamocortical neurons on the IA-lesioned side. We speculate that as the result of unilateral striatal lesion, a latent thalamocortical imbalance develops. In untreated animals, the intact side may provide compensation. If the thalamocortical tonus on

stedt, 1984). They may be largely separated on the neurons of direct and indirect striatofugal pathways, respectively (Gerfen, 1992). It is known that stimulation of the dopamine receptors modulates the activity of the motor circuit of the basal ganglia represented by two major pathways: transmission over the direct pathway is facilitated via dopamine D₁ receptors, and transmission over the indirect pathway is inhibited via dopamine D₂ receptors. Phasic activation of the direct pathway results in the reduction of tonic inhibitory basal ganglia output, resulting in the disinhibition of thalamocortical neurons and facilitation of movement. By
the intact side is decreased due to the blockade of the dopamine D₂ receptors, the increased thalamocortical tonus on the lesioned side is disclosed, leading to contralateral posture of the animals.

In kainic acid-lesioned animals, neuroleptic agents inhibited the ipsilateral turning induced by apomorphine, whereas the induction of contralateral turning was not described

(Schwarcz et al., 1979; Herrera-Marschitz and Ungerstedt, 1984). On the other hand, our experiments in IA-lesioned animals pretreated with haloperidol have shown that the stimulation of dopamine D₁ receptors by SKF-82958 induces a weak contralateral turning. It appears as if the dopamine D₂ receptor blockade determines the side of rotation, whereas the dopamine D₁ receptor stimulation determines the locomotor component (frequency of rotation), implying some similarities with the concept of a two-component model of rotating rodent (Kelly, 1977; Pycock and Marsden, 1978). The same explanation could be used to explain the effects of LEK-8829 as an D₁ agonist/D₂ antagonist drug. The intensity of the LEK-8829-induced contralateral turning may, however, also depend on additional receptor affinities of the drug (blockade of receptors 5-HT₁A and/or 5-HT₂, cf. Krisch et al., 1994, 1996).

Interaction of LEK-8829 with Bromocriptine. The treatment with a high dose of bromocriptine induced a prolonged ipsilateral turning of low frequency that was blocked by haloperidol. Low doses of LEK-8829 significantly potentiated the ipsilateral turning induced by a high dose of bromocriptine. The potentiation of bromocriptine-induced turning by LEK-8829 suggests the synergistic effects of bromocriptine and LEK-8829 on dopamine D₁ and D₂ receptors, respectively. The synergism of LEK-8829 with bromocriptine was prevented either by SCH-23390 or by haloperidol. It was described previously that dopamine D₁ receptor agonists act synergistically with bromocriptine (Robertson and Robertson, 1986; Weick and Walters, 1987; Robertson et al., 1992). The ipsilateral turning mediated by dopamine D₂ receptor agonists, such as bromocriptine, may therefore depend on the coactivation of dopamine D₁ receptors by endogenous dopamine (Dziewczapolski et al., 1997). By analogy, the blocking effect of haloperidol on the potentiation of bromocriptine-induced ipsilateral turning in response to LEK-8829 may also be explained by the necessity of the costimulation of dopamine D₂ receptors for the induction of significant ipsilateral turning by the drugs acting on dopamine D₁ receptors in this model.

The potentiation of bromocriptine-induced ipsilateral turning in response to LEK-8829, however, contradicts the previous findings (Krisch et al., 1994) regarding the antagonistic effect of LEK-8829 on dopamine D₂ receptors; namely, if LEK-8829 is an antagonist of dopamine D₂ receptors, the drug should block the agonist effects of bromocriptine at dopamine D₂ receptors and therefore inhibit the ipsilateral turning. This apparent contradiction was resolved in the dose-response experiments that have demonstrated the importance of the ratio of doses of both drugs with the proposed opposing activity at D₂ receptors for the synergistic potentiation of ipsilateral turning. The ipsilateral turning mediated by a high dose of bromocriptine was potentiated only by the lower doses of LEK-8829, indicating that the low dose of LEK-8829 may not prevent the stimulation of dopamine D₂ receptors by a high dose of bromocriptine. On the contrary, our experiments have demonstrated that it may in fact induce synergistic effects by costimulating dopamine D₁ receptors. When the highest dose of LEK-8829 was used, the potentiation of ipsilateral turning occurred only for a brief period of time and was followed by a longer period of inhibition of ipsilateral turning. This was followed by the gradual disinhibition of ipsilateral turning and switch to contralat-
eral turning toward the end of the experiment. The complex turning profile observed in animals pretreated with a high dose of bromocriptine in response to a high dose of LEK-8829 may be dependent on the pharmacokinetic factors determining the rate of displacement of bromocriptine from D2 receptors by LEK-8829. We speculate that during the initial displacement period, the dopamine D1 and D2 receptors are costimulated by LEK-8829 and bromocriptine, respectively, synergistically potentiating the ipsilateral turning behavior for a brief period of time. Due to the gradual displacement of bromocriptine from the dopamine D2 receptors by LEK-8829, the stimulation of dopamine D2 receptors eventually becomes insufficient for the synergistic costimulation of dopamine D1 receptors, resulting in the gradual weakening of ipsilateral turning. When the displacement of bromocriptine is completed, the resulting blockade of D2 receptors and stimulation of D1 receptors by LEK-8829 result in contralateral turning. However, this explanation does not explain the apparent disinhibition of ipsilateral turning in the period preceding the onset of contralateral turning.

Our results have shown that LEK-8829 may be an agonist of dopamine D1 receptors and an antagonist at dopamine D2 receptors in dopamine-innervated striatum. This finding is in agreement with our results in the 6-OHDA model, in which LEK-8829 was found to be an agonist on dopamine D1 and an antagonist on dopamine D2 receptors in dopamine-depleted striatum (Zivin et al., 1996, 1998).

The recent proposals that neuroleptic agents with additional D1 agonistic properties may be beneficial in the treatment of the negative symptoms of schizophrenia (Kahn and Davis, 1995; Okubo et al., 1997; Lidow et al., 1998) put LEK-8829, with its D1 agonistic and D2/5-HT2 antagonistic properties, in a promising perspective.

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