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Short communication

Antiparkinsonian potential of interaction of LEK-8829 with bromocriptine

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Abstract

The ergoline derivative, LEK-8829 (9,10-didehydro-*N*-methyl-(2-propynyl)-6-methyl-8-aminomethylergoline), has been proposed as a potential atypical antipsychotic drug with antagonistic actions at dopamine D_2 and serotonin 5-HT_2 and 5-HT_{1A} receptors (Krisch et al., 1994, 1996). LEK-8829 also induces contralateral turning in rats with 6-hydroxydopamine-induced unilateral lesion of dopamine nigrostriatal neurons. Turning is blocked by SCH-23390 (*R*(+)-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine), a dopamine D_1 receptor antagonist. It has been suggested that LEK-8829 could have beneficial effects in parkinsonian patients suffering from psychotic episodes induced as a side-effect of antiparkinsonian treatment with dopamine D_2 receptor agonists. Therefore, we now investigated the interaction of LEK-8829 with the dopamine D_2 receptor agonist bromocriptine (2-bromo-α-ergokryptine) in 6-hydroxydopamine-lesioned rats. Treatment with either LEK-8829 (3 mg kg^{-1}) or bromocriptine (3 mg kg^{-1}) induced a vigorous contralateral turning response. The cumulated number of turns induced by the treatment with both drugs combined was not significantly different from the cumulated number of turns induced by single-drug treatment. The pretreatment with SCH-23390 (1 mg kg^{-1}) did not have a significant effect on the bromocriptine-induced turning but significantly decreased the turning observed after the combined LEK-8829/bromocriptine treatment. We conclude that in the 6-hydroxydopamine model, the turning behaviour mediated by the LEK-8829/bromocriptine combination may be the result of opposing activity of both drugs at dopamine D_2 receptors with concomitant stimulation of dopamine D_1 receptors by LEK-8829. Therefore, LEK-8829 may have a potential for the therapy of parkinsonism complicated by dopamine D_2 receptor agonist drug-induced psychosis.

Keywords: LEK-8829; Bromocriptine; 6-Hydroxydopamine; Turning behaviour; Dopamine receptor; Parkinsonism

1. Introduction

Rats with unilateral dopaminergic deafferentation of the striatum after injection of 6-hydroxydopamine in the medial forebrain bundle (Ungerstedt, 1971) can be used to predict the antiparkinsonian activity of directly acting dopaminergic drugs. The 6-hydroxydopamine-lesioned rats develop a supersensitive response of dopaminoceptive neurons in the dopamine-depleted striatum (Ungerstedt, 1971). In this model, the direct dopamine receptor agonists induce a rotational behaviour toward the non-lesioned side (contralateral rotation).

Bromocriptine (2-bromo-α-ergokryptine) is a dopamine D_2 receptor agonist, used in the treatment of parkinsonism (Calne et al., 1974; Lees et al., 1978; Montastruc et al., 1993). The drug induces contralateral turning in 6-hydroxydopamine-lesioned rats. Its dopamine D_2 receptor stimulating activity can be antagonized with dopamine D_2 receptor antagonists (Johnson et al., 1976; Heal et al., 1980; Karlsson et al., 1988) and depends partially on the coactivation of dopamine D_1 receptors (Jackson et al., 1988).

In a study on intact rats, LEK-8829 (9,10-didehydro-*N*-methyl-(2-propynyl)-6-methyl-8-aminomethylergoline) was found to be an antagonist of dopamine D_2 receptors and of serotonin 5-HT_2 and 5-HT_{1A} receptors, and was designed as a potential atypical antipsychotic drug (Krisch et al., 1994, 1996). Additionally, the drug induces dose-dependent contralateral turning in 6-hydroxydopamine-lesioned rats and induces c-*fos* mRNA in dopamine-depleted striatum. Both effects of LEK-8829 can be antagonized by

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SCH-23390 (*R*(+)-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine), a selective dopamine D_1 receptor antagonist. The selective inhibition of LEK-8829-induced contralateral turning by SCH-23390 but not by haloperidol indicates an agonistic action of LEK-8829 on dopamine D_1 receptors (Živin et al., 1996). It has been proposed that, due to potential antipsychotic properties of LEK-8829 combined with the agonistic activity at dopamine D_1 receptors, the drug could be potentially useful in the treatment of parkinsonian patients who develop psychotic symptoms as a side-effect of antiparkinsonian therapy with dopamine D_2 receptor agonists (Živin et al., 1996).

The aim of the present study was to investigate if LEK-8829 retains its dopamine D_2 receptor antagonist activity in the animals with dopamine depleted striatum. We therefore investigated the effect of LEK-8829 on the turning behaviour induced by the dopamine D_2 receptor agonist, bromocriptine, in the 6-hydroxydopamine-lesioned rats. We used selective antagonists of dopamine D_1 and D_2 receptors (SCH-23390 and haloperidol, respectively) to characterize the receptor mechanisms involved in the turning behaviour mediated by the combined treatment with bromocriptine and LEK-8829.

2. Materials and methods

2.1. Animals

We used male Wistar rats. The animals were maintained on a 12-h light–dark cycle (light on: 0700–1900 h) in a temperature-controlled colony room at 22–24°C with free access to rodent pellets and tap water. They were housed in groups of four in standard plastic cages with sawdust cover on the floor throughout the experiment.

2.2. Drugs

The following drugs were used: apomorphine hydrochloride (RBI, Natick, MA, USA) was dissolved in 0.9% saline containing 0.02% ascorbic acid; 9,10-didehydro-*N*-methyl-(2-propynyl)-6-methyl-8-aminomethylergoline bimaleinate (LEK-8829; LEK, Ljubljana, Slovenia) was dissolved in 0.9% saline; haloperidol (Haldol amp. 5 mg ml^{-1}; Krka-Jenssen, Novo Mesto, Slovenia) was diluted with 0.9% saline; *R*(+)-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrochloride (SCH-23390; RBI, Natick, MA, USA) and 2-bromo-α-ergokryptine methanesulfonate (bromocriptine; LEK) were dissolved in dimethylsulfoxide (DMSO), the final solution being made up with 0.9% saline and DMSO (2:1). The doses refer to the form indicated above except for LEK-8829, that was calculated as the base. The drugs were administered s.c. in a volume of 2 ml kg^{-1}, except for bromocriptine that was injected i.p.

2.3. 6-Hydroxydopamine lesions of the nigrostriatal pathway

The stereotaxic lesions were performed on experimentally naive rats, weighing between 150–200 g. The animals were deeply anesthetized with the i.p. injection of Rompun® (Bayer, Leverkusen, Germany; 8 mg kg^{-1}), Ketanest® (Parke Davies, Wien, Austria; 60 mg kg^{-1}), and atropine (Belupo, Koprivnica, Croatia; 0.6 mg kg^{-1}) and were placed in a stereotaxic frame (TrentWells, South Gate, CA, USA). They received 6-hydroxydopamine hydrobromide (RBI, Natick, MA, USA; 8 μg of free base dissolved in 0.9% saline containing 0.02% ascorbic acid) infused at a rate of 1 μl min^{-1} over 4 min into the right medial forebrain bundle: A = 3 mm, L = 1.2 mm and V = 7.3 mm; anterior coordinate from lambda, lateral coordinate from the midline, ventral coordinate from the surface of the dura (stereotaxic coordinates; 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, USA). The injection cannula was retracted 2 min after the termination of infusion. Following surgery, the lesioned animals were left to recover for 28 days, and to allow for neuronal degeneration.

2.4. Recording of rotational behaviour

Each rat was placed in a plastic cylindrical chamber (40 cm diameter) of the Lablinc automated rotameter system (Colbourn Instruments, Allentown, PA, USA) designed for the electromechanical recording (Ungerstedt and Arbuthnott, 1970) of the rotational behaviour of eight animals simultaneously. The data files of the turning profiles of each animal (i.e., the full left/right turns per min) recorded by the L2T2S data acquisition software (Colbourn Instruments) were graphically represented and analyzed using a standard Lotus 1-2-3 spreadsheet, running on a personal computer.

2.5. Apomorphine test

To determine the development of dopaminergic denervation supersensitivity and to stabilize the rotational response, the 6-hydroxydopamine-lesioned animals were primed for stimulation of dopamine D_1 and D_2 receptors by the treatment with apomorphine hydrochloride (0.05 mg kg^{-1}) in the fifth and sixth post-operative weeks. Only the apomorphine-primed 6-hydroxydopamine-lesioned rats responding with at least 150 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 were performed one week after the second priming session with apomorphine.

2.6. Experimental protocols

The time of drug injections for the characterization of bromocriptine/LEK-8829 interactions was determined in a series of preliminary experiments. In the preliminary experiment, the latency to the onset of bromocriptine-induced turning was long (51 ± 22 min), as compared to the short latency to the onset of LEK-8829-induced turning (3 ± 1 min). LEK-8829 was therefore injected 50 min after bromocriptine. The antagonist drugs were given 20 min before the injection of LEK-8829.

In the first experiment, six groups of 6-hydroxydopamine-lesioned animals were used (groups A–F). Each group was treated in two experimental sessions, with 1 week of drug-free period between the sessions. Group A ($n = 4$) received injections of saline at 0 min and 30 min, followed by LEK-8829 (3 mg kg^{-1}) at 50 min. In the second experimental session, the rats were given saline at 0 min, followed by SCH-23390 (1 mg kg^{-1}) at 30 min and LEK-8829 (3 mg kg^{-1}) at 50 min.

Groups B–F received injections of bromocriptine (3 mg kg^{-1}) at 0 min, followed by saline at 30 min and at 50 min in the first experimental session. In the second experimental session, the groups received three injections given at 0, 30 and 50 min as follows: group B ($n = 7$) was injected with bromocriptine (3 mg kg^{-1}), saline and LEK-8829 (3 mg kg^{-1}), group C ($n = 4$) was injected with bromocriptine (3 mg kg^{-1}), SCH-23390 (1 mg kg^{-1}) and saline, group D ($n = 7$) was injected with bromocriptine (3 mg kg^{-1}), SCH-23390 (1 mg kg^{-1}) and LEK-8829 (3 mg kg^{-1}), group E ($n = 4$) was injected with bromocriptine (3 mg kg^{-1}), saline and haloperidol (0.5 mg kg^{-1}) and group F ($n = 4$) was injected with bromocriptine (3 mg kg^{-1}), SCH-23390 (1 mg kg^{-1}) and haloperidol (0.5 mg kg^{-1}).

In a second experiment, a group of four animals was treated in three experimental sessions performed at weekly intervals: (1) bromocriptine (0.25 mg kg^{-1}) at 0 min, followed by saline at 50 min; (2) saline at 0 min followed, by LEK-8829 (0.01 mg kg^{-1}) at 50 min; and (3) bromocriptine (0.25 mg kg^{-1}) at 0 min, followed by LEK-8829 (0.01 mg kg^{-1}) at 50 min.

2.7. Statistical analysis

All values in the figures and text are expressed as means ± S.E.M. of the number of turns during the observation period, where *n* represents the number of animals. For the first experiment, the control values (i.e., the mean cumulated number of contralateral turns recorded in an 8-h observation period induced with either LEK-8829 (group A) or with bromocriptine (groups B–F) were obtained in the first experimental session. The effects of drug treatments on the contralateral turning induced with LEK-8829 (group A) or with bromocriptine (groups B–F) were then evaluated separately for each group by comparing the mean cumulated number of contralateral turns recorded in the second experimental session to the control values recorded in the first experimental session. The statistical significance of the effects was calculated for each group of animals, using the paired, two-tailed Student's *t*-test. To evaluate if there was a significant difference in the effects of different treatments among experimental groups, a One-way analysis of variance (ANOVA) was performed for groups A–F, separately for the first and for the second experimental session. Tukey's post-hoc HSD Multiple Comparison Test was then used to evaluate which treatments in the first or in the second experimental session were significantly different. One-way ANOVA was also used for the statistical evaluation of the second experiment. The alpha level for the ANOVA procedure was set at 0.05.

3. Results

In the first experiment, we characterized the interaction of bromocriptine (3 mg kg^{-1}) and LEK-8829 (3 mg kg^{-1}) at dopamine D_1 and D_2 receptors. In the first experimental session with group A, the (saline + saline + LEK-8829) treatment yielded the profile of LEK-8829-induced turning. Saline injections did not induce any significant turning. There was a short latency to the onset of contralateral turning after injection of LEK-8829 (3 ± 1 min). A distinctive peak of rotational frequency (27 ± 7 turns min^{-1}) was observed shortly after the administration of the drug (Fig. 1A). There were 5050 ± 522 turns ($n = 4$) recorded in 8 h from the start of the experimental session (Fig. 2A). In the second experimental session, the (saline + SCH-23390 + LEK) treatment revealed the effect of dopamine D_1 receptor blockade on the profile of LEK-8829-induced turning. The treatment with SCH-23390 (1 mg kg^{-1}) almost completely blocked the cumulated number of turns induced by LEK-8829 (Fig. 1A and Fig. 2A).

The first experimental sessions with groups B, C, D, E and F that received the (bromocriptine + saline + saline) treatment, yielded the profile of bromocriptine-induced turning behaviour. The treatment with bromocriptine (3 mg kg^{-1}) induced intensive contralateral turning behaviour ($n = 26$, 5470 ± 334 turns). The turning was of long duration (> 12 h), with the delayed onset at 40 ± 8 min after the injection of bromocriptine ((bromocriptine + saline + saline) profiles, Fig. 1B–F). The second experimental session performed on the same groups of animals was used to characterize the following drug interactions:

Group B received the (bromocriptine + saline + LEK-8829) treatment to investigate the effect of LEK-8829 on the profile of bromocriptine-induced turning. The administration of LEK-8829 50 min after the injection of bromocriptine did not significantly affect the profile of apparently bromocriptine-induced contralateral turning, ex-

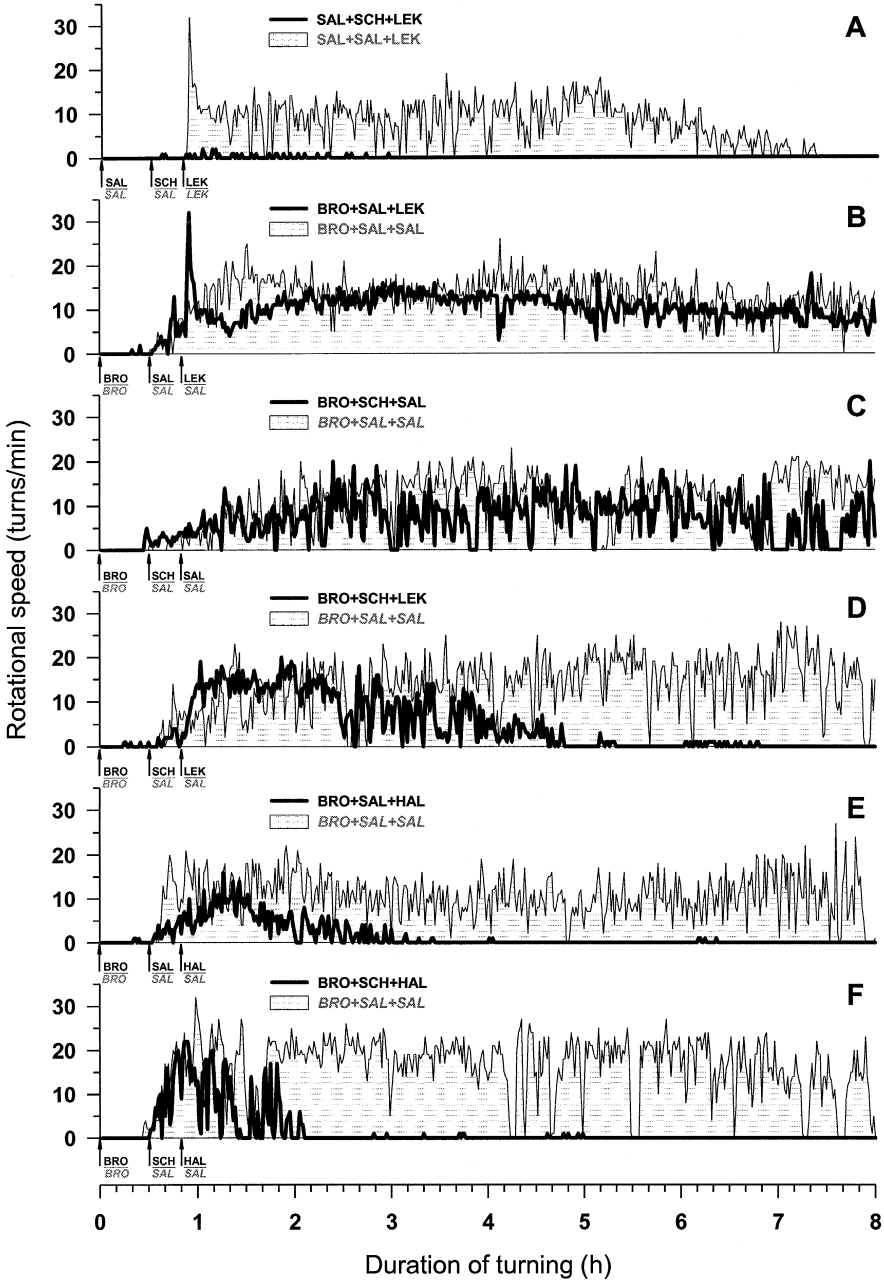

Fig. 1. The effect of SCH-23390 and haloperidol on the turning profile for bromocriptine: LEK-8829 interaction in 6-hydroxydopamine-lesioned rats. Each panel (A–F) represents the turning profile of an individual rat in the first experimental session (control session, shown by dotted profile) as compared to the turning profile of the same rat during the second experimental session after one week drug-free (shown by black line). In the first experimental session, group A was treated with saline (SAL) at 0 min and at 30 min, followed by the injection of LEK-8829 (3 mg kg^{-1} (LEK) at 50 min, whereas groups B–F were treated with bromocriptine (3 mg kg^{-1}) (BRO) at 0 min, followed by saline (SAL) at 30 and 50 min. In the second experimental session, the groups were treated with drug/saline injections at 0, 30 and 50 min according to the following protocols: group A received saline, SCH-23390 (1 mg kg^{-1}) and LEK-8829 (3 mg kg^{-1}) (SAL + SCH + LEK); group B received bromocriptine (3 mg kg^{-1}), saline, LEK-8829 (3 mg kg^{-1}) (BRO + SAL + LEK); group C received bromocriptine (3 mg kg^{-1}), SCH-23390 (1 mg kg^{-1}) and saline (BRO + SCH + SAL); group D received bromocriptine (3 mg kg^{-1}) SCH-23390 (1 mg kg^{-1}) and LEK-8829 (3 mg kg^{-1}) (BRO + SCH + LEK); group E received bromocriptine (3 mg kg^{-1}) saline and haloperidol (0.5 mg kg^{-1}) (BRO + SAL + HAL); group F received bromocriptine (3 mg kg^{-1}) SCH-23390 (1 mg kg^{-1}), haloperidol (0.5 mg kg^{-1}) (BRO + SCH + HAL). Note the peak of rotational frequency after the injection of LEK-8829 (A, B), and the effects of the blockade of the dopamine D_1 and/or D_2 receptors on the profiles of turning behaviour induced by LEK-8829 (A) or by bromocriptine (C, E, F). The effect of the blockade of dopamine D_1 receptors on the turning profile for bromocriptine: LEK-8829 intersection is shown in panel D.

cept for the occurrence of a distinctive peak of rotatory speed observed shortly after the injection of LEK-8829 (Fig. 1B). There was a small reduction of the cumulated number of turns as compared to that in the control experiment, although it did not reach statistical significance (by −23% as compared to the control (bromocriptine + saline

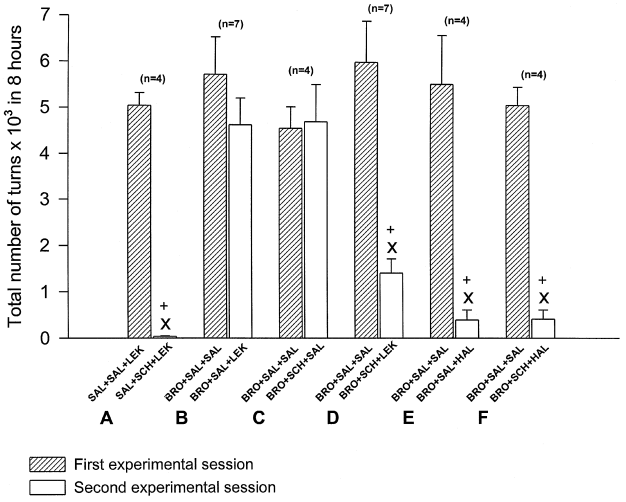

Fig. 2. The effect of SCH-23390 and haloperidol on the turning profile for bromocriptine/LEK-8829 interaction in 6-hydroxydopamine-lesioned rats. In the first experimental session (control session, represented by the hatched columns), group A was treated with saline (SAL) at 0 min and at 30 min, followed by the injection of LEK-8829 (3 mg kg^{-1}) at 50 min, whereas groups B–F were treated with bromocriptine (3 mg kg^{-1}) (BRO) at 0 min followed by saline at 30 and 50 min. In the second experimental session (represented by the empty columns), the groups were treated with drugs/saline injections at 0, 30 and 50 min according to the following protocols: group A received saline, SCH-23390 (1 mg kg^{-1}) and LEK-8829 (3 mg kg^{-1}) (SAL + SCH + LEK); group B received bromocriptine (3 mg kg^{-1} saline, LEK-8829 (3 mg kg^{-1}) (BRO + SAL + LEK); group C received bromocriptine (3 mg kg^{-1}), SCH-23390 (1 mg kg^{-1}) and saline (BRO + SCH + SAL); group D received bromocriptine (3 mg kg^{-1}), SCH-23390 (1 mg kg^{-1}) and LEK-8829 (3 mg kg^{-1}) (BRO + SCH + LEK); group E received bromocriptine (3 mg kg^{-1}), saline and haloperidol (0.5 mg kg^{-1}) (BRO + SAL + HAL); group F received bromocriptine (3 mg kg^{-1}), SCH-23390 (1 mg kg^{-1}), haloperidol (0.5 mg kg^{-1}) (BRO + SCH + HAL). Columns represents mean cumulated contralateral turns recorded for 8 h after administration of bromocriptine or saline, error bars indicate S.E.M. The number of animals in each group is indicated in the brackets above each pair of columns. Statistical evaluation: (+), significantly decreased cumulated number of turns in the second experimental session (groups A, D, E and F) as compared to the mean cumulated number of turns in the corresponding matched control session ($P < 0.01$, paired, two-tailed Student's *t*-test). (X), significantly different mean cumulated number of turns in the second experimental session in groups A, D, E, F, as compared to groups B and C (One-way ANOVA with Tukey's HSD Multiple Comparison Test, $P < 0.05$).

+ saline) session, $n = 7$, $P = 0.21$, paired, two-tailed Student's *t*-test) (Fig. 2B). Group C received (bromocriptine + SCH-23390 + saline) treatment to study the effect of dopamine D_1 receptor blockade on bromocriptine-induced turning. The cumulated number of turns and the profiles of rotational speed were not significantly different from the corresponding turning values recorded in the control experiment (Figs. 2 and 1C, respectively). Group D received the (bromocriptine + SCH-23390 + LEK-8829) treatment in order to study the effect of the blockade of dopamine D_1 receptors on the bromocriptine/LEK-8829 interaction. The group exhibited a significant reduction of the cumulated number of turns (by −77%, as compared to the control (bromocriptine + saline + LEK-8829) session, $n = 7$, $P < 0.01$, paired, two-tailed Student's *t*-test) (Fig. 2D). The analysis of the turning profiles in this group showed, that the administration of SCH-23390 prevented the occurrence of the typical rotational peak that characterized the turning profile of the (bromocriptine + saline + LEK-8829) session with group B. The reduction of the cumulated number of turns was mainly due to the considerable reduction of the duration of turning (Fig. 1D). Groups E and F received the (bromocriptine + saline + haloperidol) and (bromocriptine + SCH-23390 + haloperidol) treatment, respectively, to study the effects of the blockade of dopamine D_2 receptors (by haloperidol 0.5 mg kg^{-1}) or the blockade of dopamine D_1 and D_2 receptors (by combination of haloperidol 0.5 mg kg^{-1} and SCH-23390 1 mg kg^{-1}) on the bromocriptine-induced turning. Both groups exhibited a significant reduction of the cumulated number of turns (by −93% $n = 4$, $P < 0.01$, paired, two-tailed Student's *t*-test) and by −92% ($n = 4$, $P < 0.01$, paired, two-tailed Student's *t*-test), respectively, as compared to the corresponding control (bromocriptine + saline + saline) sessions (Fig. 2E and F). The cumulative number of turns was reduced, mainly because of the considerable shortening of the duration of bromocriptine-induced turning behaviour (Fig. 1E and F).

One-way ANOVA showed that there were no significant differences among the mean cumulated contralateral turns among groups A–F in the first experimental session in which the contralateral turning was induced either with 3 mg kg^{-1} of LEK-8829 (group A, receiving (saline + saline + LEK-8829) treatment) or with 3 mg kg^{-1} of bromocriptine (groups B, C, D, and F, receiving (bromocriptine + saline + saline) treatment). On the other hand, there were statistically significant differences in the effects of drug treatments on the mean cumulated number of turns induced with LEK-8829 and/or bromocriptine in groups A–F in the second experimental session. Tukey's HSD Multiple Comparison Test showed that there was a significantly lower mean cumulated number of turns in treatment groups A (receiving (saline + SCH-23390 + LEK)), D (receiving (bromocriptine + SCH-23390 + LEK-8829)), E (receiving (bromocriptine + saline + haloperidol)) and F (receiving (bromocriptine + SCH-23390 + haloperidol)) as compared to the treatment groups B (receiving (bromocriptine + saline + LEK-8829)) or C (receiving (bromocriptine + SCH-23390 + saline)).

In the second experiment, a group of four animals was used to assess the interaction of low dose of bromocriptine (0.25 mg kg^{-1}) with a low dose of LEK-8829 (0.01 mg kg^{-1}) that per se induced only a few number of contralateral turns per session (5 ± 2 and 18 ± 13, respectively). The statistical analysis, using One-way ANOVA, did not show a significant change in the mean cumulated number of turns as a result of combined treatment with the above doses of both drugs (6 ± 8 contralateral turns per session).

4. Discussion

In the 6-hydroxydopamine model, the selective agonists of either dopamine D_1 or D_2 receptors induce an intense rotational behaviour that is sensitive only to inhibition by the antagonists of the respective dopamine receptor subtype (Sonsalla et al., 1988; Waddington and Daly, 1993). For example, LEK-8829 induces a dose-dependent contralateral rotational behaviour in 6-hydroxydopamine-lesioned rats that is antagonized by SCH-23390, a selective antagonist of dopamine D_1 receptors, but not by haloperidol, a selective antagonist of dopamine D_2 receptors (Živin et al., 1996). On the other hand, bromocriptine-induced contralateral turning behaviour in the 6-hydroxydopamine model is antagonized only by selective dopamine D_2 receptor antagonists (Johnson et al., 1976; Heal et al., 1980; Karlsson et al., 1988). This observation was confirmed in our experiments, since the bromocriptine-induced contralateral turning in 6-hydroxydopamine-lesioned animals was blocked by haloperidol, but not by a high dose of SCH-23390. The contralateral turning response to selective dopamine D_1 or D_2 receptor agonists in 6-hydroxydopamine-lesioned animals may be explained by the development of a supersensitive response by dopaminoceptive mechanisms (Ungerstedt, 1971) and the development of uncoupling of functional cooperativity between striatal dopamine D_1 and D_2 receptors in dopamine-depleted striatum (Sonsalla et al., 1988; Hu et al., 1990). Despite the uncoupling of functional cooperativity, the specific dopamine D_1 and D_2 receptor agonists still have a synergistic action on the rotational behaviour in 6-hydroxydopamine-lesioned animals (Robertson and Robertson, 1986; Rouillard and Bedard, 1988). On the other hand, the stimulation of dopamine D_1 receptors with concomitant blockade of dopamine D_2 receptors could even increase the rotational response, as shown by Karlsson et al. (1988). In their experiments in 6-hydroxydopamine-lesioned rats, the dopamine D_2 receptor antagonist, sulpiride, potentiated the rotational behaviour induced by the dopamine D_1 agonist, CY 208–243 ((−)-4,6,6a,7,8,12b-hexahydro-7-methyl-indolo[4,3-*ab*]phenanthridine). It has been shown previously that the pretreatment with haloperidol does not significantly affect the contralateral turning induced with LEK-8829 in the 6-hydroxydopamine model (Živin et al., 1996). Since the 6-hydroxydopamine-lesioned animals exhibit a vigorous contralateral turning behaviour when challenged with LEK-8829, it may be assumed, that the blockade of dopamine D_2 receptors with LEK-8829 does not significantly weaken the dopamine D_1 receptor-mediated contralateral rotational response induced by LEK-8829 in this model.

The rotational behaviour induced by LEK-8829 could in principle be modulated also by the interaction of the drug with serotonin receptors (Gerber et al., 1988) and glutamate receptors (Starr, 1995). The partial agonism at serotonin 5-HT_{1A} receptors could contribute to the contralateral rotations induced by LEK-8829. This mechanism of rotation was excluded by checking the LEK-8829-induced rotatory response in rats pretreated with pindolol (serotonin 5-HT_{1A} receptor antagonist) (Živin et al., 1996). The possible modulation of rotational behaviour by the action of LEK-8829 at different subtypes of glutamate receptors may be excluded on the basis of the in vitro binding experiments, since the drug does not exhibit any affinity to the *N*-methyl-D-aspartate (NMDA), quisqualate or kainate subtype of glutamate receptors (Panlabs).

In the present experiments, the combined treatment of 6-hydroxydopamine-lesioned animals with LEK-8829 and bromocriptine in doses that per se induced only a weak contralateral rotational response, did not produce any potentiation of turning behaviour. This was not unexpected, since it may be explained by the blockade of the effect of bromocriptine by the dopamine D_2 receptor antagonistic activity of LEK-8829.

On the other hand, the combined treatment with both drugs using the doses that induce vigorous contralateral turning behaviour (first experiment, treatment B), resulted in a turning profile very similar to the typical turning profile mediated by the dopamine D_1 receptor activity of LEK-8829. Namely, the rotational profiles of bromocriptine/LEK-8829-treated animals were characterized by a typical peak of rotational speed that occurred shortly after the injection of LEK-8829. In contrast, the profiles of bromocriptine/saline mediated contralateral turning recorded in control experiments showed only a gradual increase in turning frequency with a long latency to the onset of turning. The initial peak of rotational speed induced by LEK-8829 may be driven by a dopamine D_1 receptor-linked mechanism, since it was prevented in rats treated with both saline/LEK-8829 and bromocriptine/LEK-8829, when the animals were pretreated with SCH-23390 20 min before the injection of LEK-8829. We therefore assumed that the contralateral turning behaviour seen in the bromocriptine/LEK-8829 interaction may be driven mainly by the dopamine D_1 receptor mechanism. The pretreatment with SCH-23390 also significantly decreased the duration of rotational behaviour induced by the combined LEK-8829/bromocriptine treatment. This implies that the antagonistic effect of LEK-8829 at dopamine D_2 receptors blocked the D_2 receptor-mediated turning behaviour driven by bromocriptine. Since there was no cumulated increase of the turning frequency in bromocriptine/LEK-8829-treated animals, this additionally supports the conclusion that the dopamine D_2 receptor blocking activity of LEK-8829 may prevent the dopamine D_2 receptor-stimulating activity of bromocriptine in animals treated with both drugs. In dopamine-depleted striatum, LEK-8829 might therefore exhibit dopamine D_1 receptor-mediated antiparkinsonian activity, blocking simultaneously the dopamine D_2 receptor overdrive induced as a possible side-effect of antiparkinsonian therapy with bromocriptine. It was shown

previously that clozapine-like properties of LEK-8829, with its antagonist activity at dopamine D_2 receptors, and at serotonin 5-HT_{1A} and 5-HT_2 receptors may be used for a potential atypical antipsychotic therapy (Krisch et al., 1994). The potential use of atypical antipsychotic clozapine as a suitable agent to treat the bromocriptine-induced psychosis, with the continued bromocriptine regimen, was reported on both previously (Scholz and Dichgans, 1985) and recently (Al-Semaan et al., 1997). We propose, therefore, that the antipsychotic activity of LEK-8829, combined with its dopamine D_1 receptor agonist activity, may be suitable to substitute for, or be used together with bromocriptine in appropriate cases. To conclude, our results with 6-hydroxydopamine-lesioned animals support the hypothesis that LEK-8829, acting as an agonist on dopamine D_1 receptors and as an antagonist on dopamine D_2 receptors, could be beneficial in the therapy of parkinsonism complicated by a psychotic reaction induced by bromocriptine.

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