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The D_1 receptor-mediated effects of the ergoline derivative LEK-8829 in rats with unilateral 6-hydroxydopamine lesions

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1 Previous experiments have suggested a potential atypical antipsychotic activity of the ergoline derivative LEK-8829. *In vitro* experiments showed a high affinity to 5-HT_{1A}, 5-HT_2 and D_2 receptors (the ratio of pK_i values 5-$HT_2/D_2 = 1.11$) and a moderate affinity to D_1 receptors. *In vivo* experiments showed antagonism of dopamine and 5-hydroxytryptamine (5-HT) receptor-linked behaviours.

2 In the present study, the rats with unilateral dopaminergic deafferentation of the striatum, induced by the lesion of the median forebrain bundle with 6-hydroxydopamine (6-OHDA), were used to determine the effects of LEK-8829 on turning behaviour and on striatal c-*fos* mRNA levels.

3 The administration of LEK-8829 induced a long lasting contralateral turning behaviour that was dose-dependent. It was found that the specific D_1 receptor antagonist SCH-23390 but not the D_2 receptor antagonist haloperidol or 5-HT_{1A} antagonist pindolol, dose-dependently inhibited the turning behaviour induced by LEK-8829.

4 In an attempt to clarify the D_1:D_2 receptor interactions involved in the action of LEK-8829 in the 6-OHDA model, we used *in situ* hybridization histochemistry to compare the effect of SCH-23390 pretreatment on striatal c-*fos* mRNA expression induced either by LEK-8829 or by the typical antipsychotic haloperidol.

5 LEK-8829 induced a bilateral striatal c-*fos* mRNA expression that was significantly higher in the denervated striatum as compared to the intact striatum and was completely blocked on both sides by pretreatment with SCH-23390. In contrast, haloperidol-induced striatal c-*fos* mRNA expression was limited to the innervated striatum and was not blocked by SCH-23390.

6 Our data demonstrate an intrinsic activity of LEK-8829 on D_1 receptors that is potentiated in the dopamine-depleted striatum. We conclude, therefore, that the putative atypical antipsychotic LEK-8829 may prove useful as an experimental tool for the study of D_1:D_2 receptor interactions and could have beneficial effects in the treatment of drug-induced psychosis in patients with Parkinson's disease.

Keywords: LEK-8829; ergoline compound; D_1 agonist; D_2 antagonist; turning behaviour; c-*fos* mRNA expression; SCH-23390; haloperidol; pindolol; striatum

Introduction

LEK-8829 is an ergoline derivative with potential antipsychotic activity. Its antipsychotic action is based on data determined *in vitro* showing a high affinity to 5-HT_2 (inhibition constant, $K_i = 5.4$ nM), D_2 ($K_i = 36.7$ nM) (Krisch *et al.*, 1994) and 5-HT_{1A} ($K_i < 10$ nM) (Panlabs) receptors and on those determined *in vivo* demonstrating antagonism of dopamine and 5-hydroxytryptamine (5-HT) receptor-linked behaviours (cataleptogenic effect in mice and rats, antagonism of apomorphine-induced locomotor activity in rats, antagonism of apomorphine-induced cage-climbing behaviour in mice, antagonism of 5-hydroxytryptophan-induced head twitch response in mice). The affinity to D_1 receptors *in vitro* was moderate ($K_i = 827$ nM) (Krisch *et al.*, 1994).

Rats with unilateral dopaminergic denervation of the striatum, induced by the lesion of the median forebrain bundle with 6-hydroxydopamine (6-OHDA), are often used for *in vivo* screening of potential dopamine (DA) agonists or antagonists. The 6-OHDA model can be utilized for the evaluation of either D_1 or D_2 receptor-driven contralateral turning behaviour that can be induced by directly acting nonselective or selective dopamine receptor agonists, after the development of a supersensitive response in the dopamine deafferentated striatum of the lesioned side (Ungerstedt, 1971a; Robertson & Robertson, 1986; Sonsalla *et al.*, 1988). Furthermore, stimulation by partial 5-HT_{1A} agonists can also induce contralateral turning in 6-OHDA-lesioned rats (Gerber *et al.*, 1988).

In the above model, the neuroleptic drugs, due to their antagonist action on D_2 receptors, inhibit the ipsilateral turning behaviour induced by the indirectly acting receptor agonist amphetamine (Ungerstedt, 1971b) and only partially decrease the intensity of contralateral turning induced by the directly acting dopamine agonist apomorphine (Sonsalla *et al.*, 1988).

In previous behavioural experiments (Krisch *et al.*, 1994) no overt signs of an intrinsic dopaminergic action of LEK-8829 could be observed. It was expected, therefore, that in rats with a unilateral lesion of the dopaminergic nigrostriatal pathway, LEK-8829 might inhibit the contralateral turning induced by apomorphine. In our preliminary experiments, however, pretreatment with LEK-8829 did not prevent the apomorphine-induced turning behaviour. On the contrary, pretreatment with 3 mg kg^{-1} s.c. of LEK-8829 *per se* induced a long lasting contralateral turning behaviour.

In the present study, with the aim to evaluate the type of receptor mechanism involved in the possible agonistic activity of LEK-8829, the LEK-8829-induced turning behaviour was analysed in the 6-OHDA model. To determine the type of receptors triggering the LEK-8829-induced turning response, the LEK-8829-induced turning behaviour was characterized by use of the D_1 antagonist SCH-23390, D_2 antagonist haloperidol and 5-HT_{1A} antagonist pindolol.

Immediate early response genes (IEGs) which are involved in the transcriptional events associated with the dopaminergic regulation of peptide neurotransmitter expression within the neostriatum are also modulated by treatment with neuroleptic drugs. Due to the specific profile of interactions with dopamine receptors, typical and atypical antipsychotics produce a differential pattern of IEGs expression in several brain regions (Nguyen *et al.*, 1992; Merchant & Dorsa, 1993; MacGibbon *et*

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al., 1994; Robertson *et al.*, 1994). It is also known that in dopamine-innervated striatum both D_2 antagonists and D_1 agonists induce an increase of striatal c-*fos* mRNA (Miller, 1990; Rogue & Vincendon, 1992; Arnauld *et al.*, 1993) with subsequent increase of striatal c-*fos* protein (Robertson *et al.*, 1989; Dragunow *et al.*, 1990; Robertson & Fibiger, 1992; Fink-Jensen & Kristensen, 1994). A synergistic action of D_1 and D_2 agonists on the turning behaviour and on the c-*fos* mRNA expression was described in the dopamine depleted striatum (Paul *et al.*, 1992). On the other hand, D_2 receptor antagonists seem to be ineffective as regards the induction of c-*fos* mRNA in the dopamine depleted striatum (Robertson & Fibiger, 1992). Since it has been claimed that D_1 receptors may be located mainly on substance P containing striatonigral neurones (Le Moine *et al.*, 1991), whereas D_2 receptors may be located mainly on the preproenkephalin A containing striatopallidal neurones (Gerfen *et al.*, 1990; Le Moine *et al.*, 1990), the pattern of striatal c-*fos* mRNA expression induced by selective D_1 agonists/D_2 antagonists in unilaterally 6-OHDA lesioned animals should reflect both, the differential distribution of dopamine receptor subtypes within striatal neuronal populations (Robertson *et al.*, 1992) and the changed sensitivity of dopamine-depleted striatum to c-*fos*-inducing dopamine drugs.

In the present study, the induction of striatal c-*fos* mRNA in rats with unilateral lesions of dopaminergic nigrostriatal neurones was studied after the application of LEK-8829 in dopamine preserved and dopamine depleted striatum. The receptor mechanism linked to the c-*fos* mRNA expression induced by LEK-8829 was analysed by use of SCH-23390 and haloperidol.

Methods

Animals

We used male Wistar rats ($n=86$). The animals were maintained on a 12 h light-dark cycle (light on: 07 h 00 min 19 - h 00 min) 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.

6-Hydroxydopamine (6-OHDA)-induced lesions of the nigrostriatal pathway

The stereotaxic lesions were performed on experimentally naive rats, weighing between 150–200 g. The animals were deeply anaesthetized with an 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 placed in a stereotaxic frame (TrentWells, basic research model, South Gate, CA, U.S.A.). 6-Hydroxydopamine HBr (RBI, Natick, U.S.A.; 8 μg of free base dissolved in 0.9% saline containing 0.02% ascorbic acid) was infused at a rate of 1 μl min^{-1} 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 and the nose bar set 2.3 mm below the interaural line. The infusion was delivered over 4 min 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, U.S.A.). The injection cannula was retracted 2 min after the infusion. Following surgery, the animals were left for 21 days to recover and to allow for neuronal degeneration.

Recording of rotational behaviour

The rats were placed in plastic bowls (30 cm in diameter) in the field of view of a video camera (up to 16 bowls). The drug-induced rotational behaviour was tape-recorded. The tapes were analysed by an event-recorder software devised in our laboratory for Lotus 1-2-3 spreadsheet, running on a PC. A trained observer (unaware of the treatment protocols), using the console keys as the interface, recorded the full left/right turns per minute, the moment of drug injection, etc. The software also allowed the time base to be adjusted according to the increased play-back speed (3 ×), shortening the event-recording time considerably.

Apomorphine test

To determine the development of dopaminergic denervation supersensitivity, the animals were challenged with apomorphine hydrochloride (0.05 mg kg^{-1}) in the fourth and in the fifth post-operative week. In order to evaluate the effect of apomorphine on LEK-8829-induced turning, a group of apomorphine-primed animals ($n=4$) and a group of apomorphine-naive animals ($n=4$) received LEK-8829 (3 mg kg^{-1}), in the sixth post-operative week. For all the subsequent experiments only the apomorphine-primed rats responding with at least 150 turns during the second apomorphine session were chosen and randomly divided into the experimental groups.

The experimental protocol for the study of LEK-8829-induced turning

The dose-response curve for LEK-8829-induced turning (i.e. total number of turns and the duration of turning behaviour) was assessed in five groups of 6-OHDA lesioned animals ($n=10$), each group receiving one of the following doses of LEK-8829: 0.09, 0.25, 0.5, 1 and 3 mg kg^{-1}. To determine the type of receptors mediating the LEK-8829-induced turning, three groups of animals were used ($n=4$). The first group was pretreated with the D_1 antagonist SCH-23390 (0.001, 0.01 and 0.05 mg kg^{-1}), the second group was pretreated with the D_2 antagonist haloperidol (0.5 and 5 mg kg^{-1}) and the third group was pretreated with the 5-HT_{1A} receptor antagonist pindolol (2 and 10 mg kg^{-1}). The pretreatment injections were administered twenty minutes before the administration of LEK-8829 (0.25 mg kg^{-1}). There was one week drug-free period between each experimental session. For each group, the effects of pretreatments with antagonists were compared to the effects of LEK-8829-only treatment (0.25 mg kg^{-1}) performed one week before and one week after the experiments with the antagonists. The reproducibility of LEK-8829-induced turning was tested in a control experiment, namely, the control group of rats ($n=3$) was treated with weekly injections of LEK-8829 (0.25 mg kg^{-1}) for five consecutive weeks.

*The experimental protocol for the study of striatal c-*fos *expression*

The striatal c-*fos* expression was studied in six groups of rats ($n=3$) receiving one of the following treatments: pretreatment with 0.9% saline followed by LEK-8829 (0.25 mg kg^{-1}), pretreatment with SCH-23390 (0.1 mg kg^{-1}) followed by LEK-8829 (0.25 mg kg^{-1}), pretreatment with haloperidol (5 mg kg^{-1}) followed by LEK-8829 (0.25 mg kg^{-1}), pretreatment with 0.9% saline followed by haloperidol (5 mg kg^{-1}), pretreatment with SCH-23390 (0.1 mg kg^{-1}) followed by haloperidol (5 mg kg^{-1}) and pretreatment with SCH-23390 (0.1 mg kg^{-1}) followed by 0.9% saline treatment. The pretreatment injections were administered twenty minutes before the treatment injections. All the animals were killed under ether anaesthesia thirty minutes after the treatment injection.

Preparation, fixation and storage of brain sections

Brains were rapidly removed and quickly frozen on dry-ice, wrapped in parafilm to prevent desiccation and stored at −80°C in a freezer until cryostat sections could be cut. Before cutting, the brains were allowed to equilibrate at −18°C in a

cryostat chamber. Coronal sections (10 μM) were cut through the neostriatum and ventral midbrain (at four evenly spaced levels between 2.2 and −0.8 from bregma and at four evenly spaced levels between −4.8 and −5.8 from bregma), then thaw mounted onto glass slides (previously baked for 4 h at 180°C and coated with 0.01% solution of (poly)L-lysine in DEPC H_2O). The sections were fixed in 4% phosphate-buffered paraformaldehyde for 5 min, washed in phosphate-buffered saline (3 changes of 1 min each), dehydrated in 70% ethanol for 5 min and stored in 95% ethanol at +4°C until processed in *in situ* hybridization histochemistry.

Oligonucleotide probes

We used an oligodeoxyribonucleotide 'antisense' probe (45 bases long) complementary to bases 471–515 of the tyrosine hydroxylase (TH) gene (Grima *et al.*, 1985), control 'sense' oligodeoxyribonucleotide probe complementary to the 'antisense' TH mRNA probe and oligodeoxyribonucleotide probe spanning amino acids 1–15 (45 mer) of the rat c-*fos* gene (Curran *et al.*, 1987). The probes were the kind gift of Dr D.J.S. Sirinathsinghji. The specificity of both 'antisense' probes was verified with Northern blot analysis of poly (A+) RNA extracted from rat brain as described previously (Sirinathsinghji *et al.*, 1994).

In situ *hybridization histochemistry*

The standard procedure described in detail by Sirinathsinghji *et al.* (1990) was performed. Briefly, the sections were removed from ethanol, allowed to dry on air and incubated with ^{35}S-labelled probe in hybridization buffer. The hybridization buffer contained 4 × SSC (1 × SSC contains 150 mM sodium chloride and 15 mM sodium citrate), 50% deionized formamide, 50 mM sodium phosphate (pH 7.0), 5 × Denhard's solution, 100 μg ml^{-1} polyadenylic acid, 10% dextran sulphate and 40 mM dithiothreitol. The oligodeoxyribonucleotide probes were labelled at the 3′ end with [^{35}S]-deoxyadenosine 5′-α-(thio)triphosphate ([^{35}S]-dATP; 1000–1500 Ci $mmol^{-1}$; DuPont NEN) and terminal deoxynucleotidyl transferase enzyme (Boehringer Mannheim, Germany) in a tailing buffer containing 500 mM K^+ cacodylate, 5 mM $CoCl_2$ and 10 μM dithiothreitol. The incubation was performed in 1 ml Eppendorf tubes for one hour in a water bath at 35°C. After the incubation, the labelled probes were purified by a spin column procedure with Sephadex G50. The specific activities of the labelled probes determined by scintillation counting ranged from 0.8 to 1.2×10^9 c.p.m. μg^{-1}. The labelled probes were used at a concentration of 3×10^3 c.p.m. μl^{-1} and 100 μl of hybridization buffer with labelled probe (i.e. 3×10^5 c.p.m.) were applied to each slide, covered with a strip of parafilm and incubated overnight (16 h) at 42°C in a humidified Petri dish. Control striatal sections were hybridized in the presence of 50 fold excess of unlabelled c-*fos* probe. Control adjacent sections at the ventral midbrain level were hybridized with the radiolabelled 'sense' TH-mRNA probe. The washing was performed for 1 h at 55°C in 1 × SSC and thereafter for one hour at room temperature. The sections were then dehydrated through an ethanol series, dried with a stream of cold air and exposed to X-ray film (Hyperfilm β-max, Amersham CEA AB, Sweden). The autoradiograms of c-*fos* and TH mRNA were exposed at room temperature for thirty days and eight days, respectively. The sections were then counterstained with methylene blue to permit the identification of brain nuclei.

Image analysis

The levels of LEK-8829-induced c-*fos* mRNA in the striatum were calculated from densitometric analysis of *in situ* hybridization X-ray film autoradiograms by use of the MCID, M4 image analyzer (Imaging Research Inc., Canada). Only the autoradiograms of the animals with no detectable TH mRNA signal at the ventral midbrain level on the 6-OHDA lesioned side were taken for the analysis of the striatal c-*fos* mRNA levels. The computer-assisted calibration of autoradiograms was performed with a series of radioactive ^{35}S-brain paste standards co-exposed to each film. The optical density (OD) of the standards was read and the fitted calibration curve representing OD as a function of the radioactivity concentration (expressed at c.p.m. per mm^2) of the standards (measured by scintillation counting at the end of exposure) was computer-calculated. There was a linear correlation between the OD of the standards and their radioactivity concentration, within the range of OD values measured on the c-*fos* mRNA autoradiograms.

The specific activity of the labelled probe (SA) was calculated as follows: SA (c.p.m. per amol) = total radioactivity of labelled probe (c.p.m.)/molar quantity of labelled probe (amol). By use of the above described protocol for labelling the 45-mer oligonucleotide probes with terminal transferase, the molar quantity of labelled probe in the spin-column eluate should be equal to the molar quantity of the probe in the tailing reaction (Wisden *et al.*, 1991). Namely, the radioactivity of the eluate (measured by scintillation counting) is contributed only by the tail of the labelled probe and virtually all the probe molecules are presumed to be labelled and recovered in the eluate. It was also determined experimentally, that in the tailing reaction, a population of molecules with different tail lengths, that presumably fit a normal distribution, is formed (Wisden *et al.*, 1991). Therefore, one is able to calculate the average SA of a population of labelled probe molecules. Molar quantities of the c-*fos* probe hybridized per unit tissue area (expressed as tissue levels of c-*fos* mRNA at amol per mm^2), were calculated from the OD values of the measured regions (converted by computer to c.p.m. per mm^2), by use of the following equation: probe bound (amol/mm^2) = c.p.m./mm^2 X $(SA)^{-1}$. Appropriate corrections were made for the decay of the SA of the probe and of the standards, taking the midpoint between apposition and development of the film as the reference time. The background autoradiographic signal (measured on the adjacent control sections where the labelled probe was hybridized in the presence of 50 fold excess of nonlabelled probe) was subtracted to correct for the non-specific autoradiographic signal. For each measurement, the digital image of the methylene blue stained section and of the corresponding autoradiogram were carefully superimposed. The outline of methylene blue stained striatum was then used as the sampling template for taking the measurement on the autoradiogram. For each animal, the average c-*fos* mRNA levels in the innervated and denervated striatum were calculated (measurements were taken from two sections at four coronal levels – see also 'Preparation, fixation and storage of brain sections').

Drugs

The following drugs were used: 9,10-didehydro-N-methyl-(2-propynyl)-6-methyl-8-aminomethylergoline bimaleinate (LEK-8829; LEK, Ljubljana, Slovenia) dissolved in 0.9% saline; apomorphine HCl (Sigma, St. Louis, MO, U.S.A.) dissolved in 0.9% saline containing 0.02% ascorbic acid; **R**(+)-SCH-23390 HCl (**R**(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepene hydrochloride, SCH-23390; RBI, Natick, USA) dissolved in dimethylsulphoxide (DMSO), the final solution being made up with 0.9% saline and DMSO (2:1); haloperidol (Haldol amp. 5 mg ml^{-1}; Krka-Janssen, Novo Mesto, Slovenia) diluted with 0.9% saline and pindolol (Sigma, Deisenhofen, Germany), dissolved in DMSO, the final solution being made up with 0.9% saline and DMSO (1:4). The doses given 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}.

Statistical analysis

The effects of apomorphine priming on LEK-8829-induced turning behaviour and the effects of SCH-23390 pretreatment on striatal c-*fos* mRNA levels induced by LEK-8829 or haloperidol were evaluated by use of unpaired, two-tailed Student's

t test. Paired, two-tailed Student's *t* test was performed for the comparisons of the striatal c-*fos* mRNA levels of the innervated versus denervated side. The dose-response effects of LEK-8829 administration, the dose-response effects of pretreatment with antagonists on LEK-8829-induced turning and the reproducibility of LEK-8829-induced turning were subjected to one-way analysis of variance (ANOVA) followed by Tukey's HSD Multiple-Comparison Test.

Results

Effect of apomorphine priming on the LEK-8829-induced turning

The comparison of LEK-8829-induced contralateral turning behaviour in a group of apomorphine-primed animals with the turning behaviour induced in a group of apomorphine-naive animals, has revealed a considerable priming effect of apomorphine on LEK-8829-induced turning. In apomorphine-primed rats the total number of turns induced by LEK-8829 (3 mg kg^{-1}) was significantly increased (by +132%; $n=4$, $P<0.01$, two-tailed Student's *t* test). The total number of turns was increased due to significant prolongation of turning behaviour (by +61%; $n=4$, $P<0.01$, two-tailed Student's *t* test) and to the increased rotational speed (by +26%; $n=4$, $P<0.01$, two-tailed Student's *t* test) of apomorphine-primed rats. The turning profile of apomorphine-primed group revealed a distinctive peak of rotational frequency (mean ± s.d. = 23 ± 7 turns), occurring shortly after the onset of turning as compared to the gradual increase of the rotational frequency observed in the turning profile of apomorphine-naive animals (Figure 1).

Effect of repeated administration of LEK-8829

Repeated weekly administration of LEK-8829 (0.25 mg kg^{-1} for five consecutive weeks) to the apomorphine-primed animals did not significantly affect the duration of LEK-8829-induced turning behaviour. Increased total number of turns was observed in the third, fourth and fifth treatment weeks, but it did not reach statistical significance ($n=3$, $P>0.05$, ANOVA with Tukey's HSD Multiple-Comparison Test) (Figure 2).

Dose-response study

LEK-8829 (0.09, 0.25, 0.5, 1 and 3 mg kg^{-1}) induced turning behaviour toward the nonlesioned side. There was a dose-dependent increase of the total number of turns and of the duration of LEK-8829-induced turning behaviour (Figure 3). On the other hand, the highest mean rotational speed was observed in a group of animals treated with the dose of 0.25 mg kg^{-1} (Figure 4). The turning profile after the lowest doses (0.09 and 0.25 mg kg^{-1}) revealed a sharp peak for increase in the rotational speed, followed by a somewhat slower decline towards the end of rotation. With the larger doses (0.5, 1 and 3 mg kg^{-1}) the initial peak rotational speed was followed by a prolonged period of intensive contralateral turning which was occasionally interrupted by periods of contralateral twisting, compulsive licking or scratching with the teeth of the contralateral fore paw and/or in the region close to the hindleg. The interfering behaviour obviously reduced the mean rotational speed and the total number of turns performed by the animals.

Effect of SCH-23390

Pretreatment with the D_1 antagonist SCH-23390, administered 20 min before the injection of LEK-8829, resulted in a dose-dependent inhibition of contralateral turning behaviour induced by LEK-8829 (0.25 mg kg^{-1}). Pretreatment with 0.001 mg kg^{-1} SCH-23390 did not significantly affect the LEK-8829-induced turning behaviour (Figure 6B). The reduction of total number of turns after pretreatment with 0.01 mg kg^{-1} SCH-23390 (by −75% as compared to the control LEK-8829-only treatment; $n=4$, $P<0.01$, two-tailed Student's *t* test) was due to increased latency to the onset of

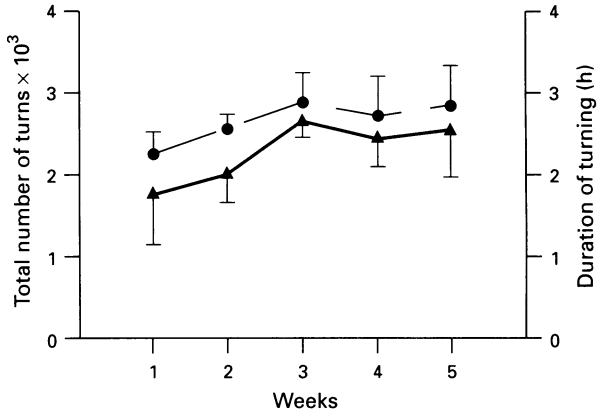

Figure 2 Total number of turns (▲) and duration of turning behaviour (●) in 6-OHDA-lesioned rats, treated at weekly intervals with LEK-8829 (0.25 mg kg^{-1}, s.c.). Means ± s.d. (vertical lines) are shown, $n=3$ in both groups. Statistical evaluation: $P>0.05$; one-way ANOVA with Tukey's HSD Multiple Comparison Test.

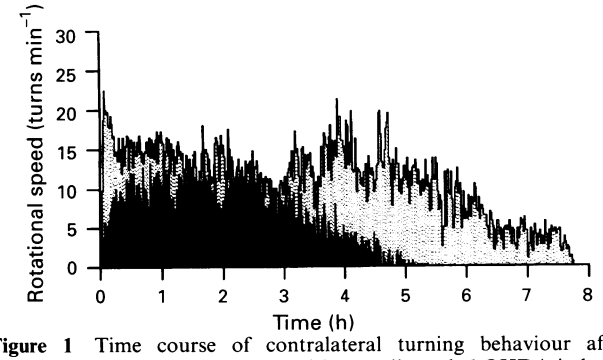

Figure 1 Time course of contralateral turning behaviour after injection of LEK-8829 in rats with a unilateral 6-OHDA-induced lesion. The two profiles show the mean rotational speed (turns min^{-1}) after administration of LEK-8829 (3 mg kg^{-1}, s.c.). Black profile: unprimed rats ($n=4$). Dotted profile: apomorphine-primed rats ($n=4$; 0.05 mg kg^{-1}, s.c., apomorphine in two consecutive weeks). Note the prolongation of the duration of turning, increased frequency of turning and a peak of high turning frequency occurring shortly after administration of LEK-8829 to the apomorphine-primed animals.

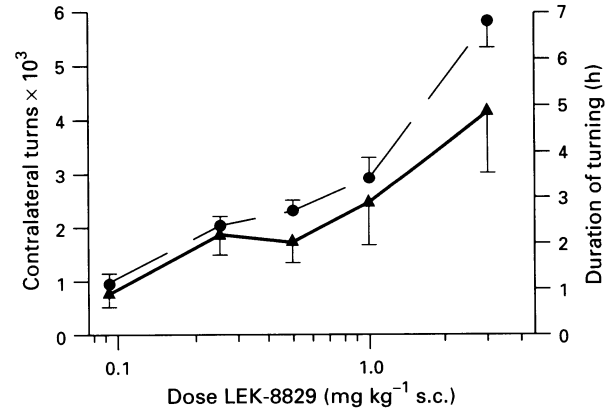

Figure 3 Dose-response curves for turning behaviour after injection of LEK-8829 (0.09, 0.25, 0.5, 1 and 3 mg kg^{-1}, s.c.) to 6-OHDA lesioned rats: (▲) total number of turns, (●) duration of turning behaviour. Means ± s.d. (vertical lines) are shown, $n=10$.

turning and to a significant reduction of the duration of turning behaviour (Figures 5a(ii) and 6C). On the other hand, pretreatment with 0.05 mg kg^{-1} SCH-23390 completely prevented the LEK-8829-induced turning behaviour (Figures 5a(iii) and 6D).

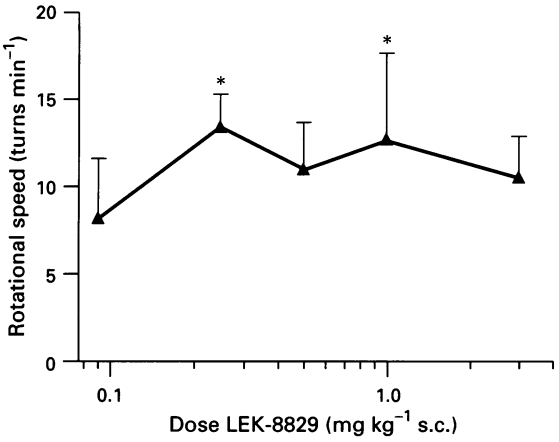

Figure 4 Dose-response curve of average rotational speed after injection of LEK-8829 (0.09, 0.25, 0.5, 1 and 3 mg kg^{-1}, s.c.) to 6-OHDA lesioned rats. Statistical evaluation: *increased mean turning frequency as compared to 0.09 mg kg^{-1} group; $n=10$; $P<0.05$, one-way ANOVA with Tukey's HSD Multiple Comparison Test.

Effect of haloperidol

Contrary to the inhibition of LEK-8829-induced turning with SCH-23390, pretreatment (20 min) with the D_2 antagonist haloperidol (0.5 mg kg^{-1}) did not significantly affect the total number of turns or the duration of turning behaviour induced by LEK-8829 (0.25 mg kg^{-1}) (Figures 5b(ii) and 7B). With the higher dose of haloperidol (5 mg kg^{-1}) the total number of contralateral turns was decreased due to the modified profile of LEK-8829-induced turning. Namely, the latency to the onset of turning was increased and the initial peak of high rotational speed was missing. In some of the animals, the contralateral turning was interrupted by a period of ipsilateral turning, which reverted to the contralateral direction, for a brief period toward the end of the turning session (Figures 5b(iii) and 7C).

Effect of pindolol

Pretreatment (20 min) with the 5-HT_{1A} antagonist pindolol (2 and 10 mg kg^{-1}) did not significantly affect the mean rotational speed, the total number of turns, the duration or the direction of turning behaviour induced by LEK-8829 (0.25 mg kg^{-1}); however, the latency to the onset of turning was increased (Figures 5c(ii) and (iii) and 8B, C).

TH mRNA expression in substantia nigra reticulata and ventral tegmental area in 6-OHDA lesioned rats

In situ hybridization histochemistry of TH mRNA was performed to select the animals showing a complete degeneration

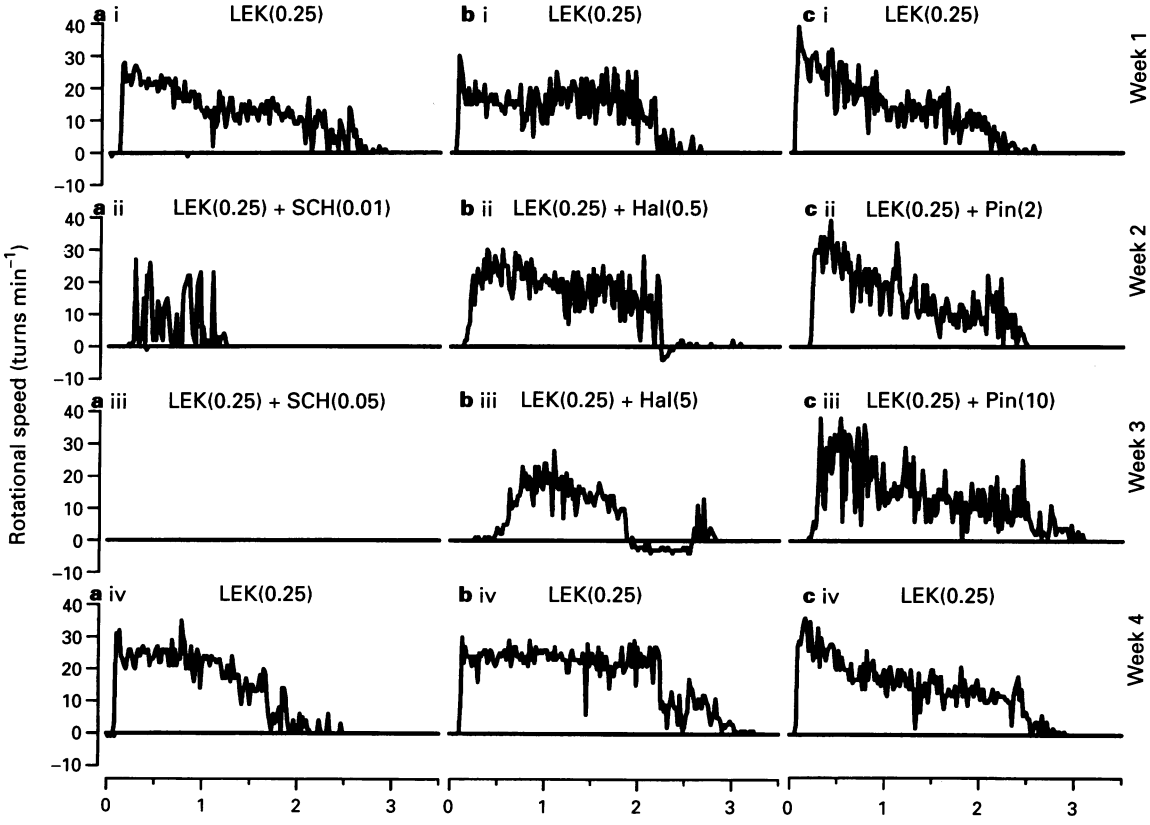

Figure 5 Individual recordings of LEK-8829-induced (0.25 mg kg^{-1}, s.c.) turning behaviour in the apomorphine-primed, 6-OHDA lesioned rats (rats a, b and c) after pretreatment (20 min) with SCH-23390 (SCH), haloperidol (Hal) or pindolol (Pin), respectively. Experimental sessions were conducted in four weekly intervals. Week 1 (ai, bi, ci) saline pretreatment; week 2: (aii) pretreatment with SCH-23390 (0.01 mg kg^{-1}, s.c.); (bii) pretreatment with haloperidol (0.5 mg kg^{-1}, s.c.); (cii) pretreatment with pindolol (2 mg kg^{-1}, s.c.); week 3: (aiii) pretreatment with SCH-23390 (0.05 mg kg^{-1}, s.c.); (biii) pretreatment with haloperidol (5.0 mg kg^{-1}, s.c.); (ciii) pretreatment with pindolol (10 mg kg^{-1}, s.c.); week 4: (aiv, biv, civ) saline pretreatment. Note the prolonged latency to the onset of turning and a shorter duration of turning behaviour after pretreatment with SCH-23390 (aii), the prolonged latency to the onset of contralateral turning and a change of the direction of turning to ipsilateral side with the reversal to the contralateral side for a short period toward the end of rotation after the pretreatment with haloperidol (bii and iii) and the prolonged latency to the onset of turning after pretreatment with pindolol (cii and iii). Above the zero line = contralateral rotations, below the zero line = ipsilateral rotations. Zero time = injection of LEK = 8829.

of dopaminergic neurones. Only the animals with a complete loss of TH mRNA signal at the film-autoradiographic level in substantia nigra compacta/ventral tegmental area were selected for the study of striatal c-*fos* mRNA expression.

*Effect of LEK-8829 on the c-*fos *mRNA expression*

The preliminary time course study of LEK-8829-induced striatal c-*fos* mRNA expression in intact animals (3 mg kg^{-1}; results not illustrated) showed a bilateral induction of striatal c-*fos* mRNA levels starting at 15 min, peaking between 30 and 45 min, declining steadily thereafter and reaching basal levels 120 min after the administration of the drug. Three rats were pretreated (20 min) with saline followed by injection of LEK-8829 (0.25 mg kg^{-1}). Thirty minutes after the injection of LEK-8829 there was a bilateral increase in striatal c-*fos* mRNA expression, with significantly higher levels of c-*fos* mRNA in the denervated striatum ($n=3$, $P<0.01$, paired Student's t test). On the intact side, the striatal c-*fos* mRNA levels exhibited a positive medio-lateral gradient, as compared to the denervated striatum where the c-*fos* mRNA levels were evenly distributed. An increase in the c-*fos* mRNA levels in the frontal region of cerebral cortex was more intense on the lesioned side. A bilateral c-*fos* expression in the primary olfactory cortex was also observed (Figures 9 and 10, Sal + LEK).

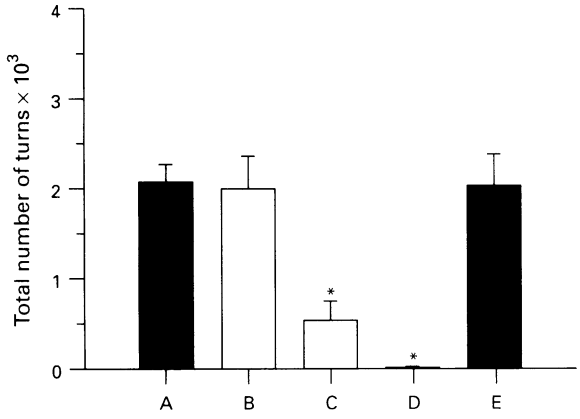

Figure 6 The effect of pretreatment with SCH-23390 on LEK-8829-induced turning in 6-OHDA-lesioned rats. Columns represent mean cumulative turns induced by LEK-8829 (0.25 mg kg^{-1}, s.c.); vertical lines show s.d., $n=4$. Experimental sessions were conducted at weekly intervals. Pretreatments: (A) saline controls, one week before sessions with the inhibitor, (B) SCH-23390 0.001 mg kg^{-1}, s.c., (C) SCH-23390 0.01 mg kg^{-1}, s.c., (D) SCH-23390 0.05 mg kg^{-1}, s.c., (E) saline controls one week after the sessions with the inhibitor. Statistical evaluation: decreased total number of turns in groups C and D as compared to saline pretreatment groups (A,E); $n=4$, $P<0.05$, one-way ANOVA with Tukey's HSD Multiple Comparison test.

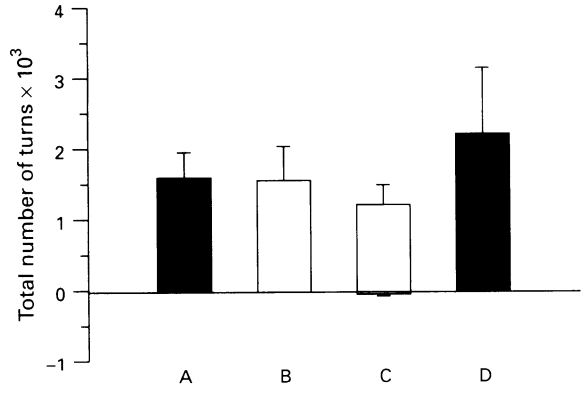

Figure 7 The effect of pretreatment with haloperidol on LEK-8829-induced turning in 6-OHDA-lesioned rats. Columns represent mean cumulative turns induced by LEK-8829 (0.25 mg kg^{-1}, s.c.); vertical lines show s.d., $n=4$. Above the zero line = contralateral turns, below the zero line = ipsilateral turns. Experimental sessions were conducted at weekly intervals. Pretreatments: (A) saline controls one week before sessions with the inhibitor, (B) haloperidol 0.5 mg kg^{-1}, s.c., (C) haloperidol 5 mg kg^{-1}, s.c., (D) saline controls one week after the sessions with the inhibitor. Statistical evaluation: there were no significant effects of haloperidol on the total number of turns (groups B and C) as compared to saline pretreatment groups (A,D); $n=4$, $P>0.05$, one-way ANOVA with Tukey's HSD Multiple Comparison test.

Three rats were pretreated (20 min) with saline followed by injection of haloperidol (5 mg kg^{-1}). Thirty minutes after the injection of haloperidol a high level of c-*fos* mRNA in the striatum of the intact side was found. No autoradiographic signal could be detected in the denervated striatum. On the other hand, there was a bilateral c-*fos* mRNA expression detected in the lateral septal nucleus and in the primary olfactory cortex. There was no increase of the c-*fos* mRNA in the frontal cortex on either side (Figures 9 and 10, Sal + Hal).

Effect of SCH-23390 on LEK-8829- and haloperidol-induced c-fos *mRNA expression*

In the group of rats pretreated (20 min) with SCH-23390 (0.1 mg kg^{-1}), there was no induction of c-*fos* mRNA by LEK-8829 (0.25 mg kg^{-1}) either in the striatum or in the frontal cortex (Figures 9 and 10, SCH + LEK). Only a low response was found bilaterally in the lateral septum and in the olfactory cortex in both hemispheres but no quantification was performed. On the other hand, pretreatment (20 min) with SCH-23390 (0.1 mg kg^{-1}) did not significantly affect the induction of c-*fos* mRNA by haloperidol (5 mg kg^{-1}) (Figures 9 and 10, SCH + Hal).

Effect of pretreatment with haloperidol on LEK-8829-induced c-fos *mRNA expression*

In the group of rats pretreated (20 min) with haloperidol (5 mg kg^{-1}) followed by the administration of LEK-8829

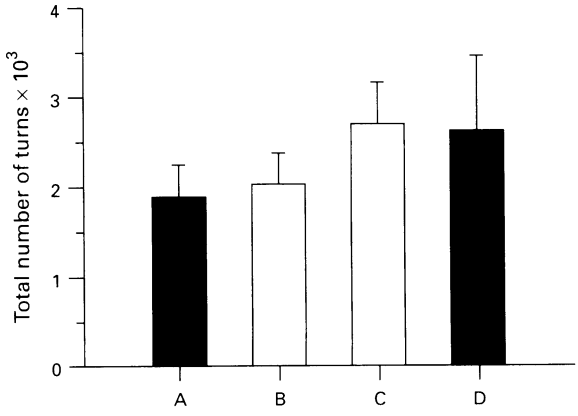

Figure 8 The effect of pretreatment with pindolol on LEK-8829-induced turning in 6-OHDA lesioned rats. Columns represent mean cumulative turns induced by LEK-8829 (0.25 mg kg^{-1}, s.c.); vertical lines show s.d., $n=4$. Experimental sessions were conducted at weekly intervals. Pretreatments: (A) saline controls one week before sessions with the inhibitor, (B) pindolol 2 mg kg^{-1}, s.c., (C) pindolol 10 mg kg^{-1}, s.c., (D) saline controls one week after the sessions with the inhibitor. Statistical evaluation: there were no significant effects of pindolol on the total number of turns (groups B and C) as compared to saline pretreatment groups (A,D); $n=4$, $P>0.05$, one-way ANOVA with Tukey's HSD Multiple Comparison test.

(0.25 mg kg^{-1}) there was a bilateral induction of c-*fos* mRNA in striatum, septum and in the primary olfactory cortex. The c-*fos* mRNA levels in the intact striatum were significantly higher ($n=3$, two tailed t test, $P<0.01$), whereas in the lesioned striatum they were not different compared to the LEK-8829-induced striatal c-*fos* mRNA levels in the saline pretreated controls. On the 6-OHDA lesioned side there was also an increase of the c-*fos* mRNA levels in the frontal cortex on the denervated side. Bilateral c-*fos* mRNA expression was found in the lateral septum and in the olfactory cortex (Figures 9 and 10, Hal + LEK).

*The c-*fos *expression in saline treated controls pretreated with SCH-23390*

Pretreatment with SCH-23390 (0.1 mg kg^{-1}), followed by treatment with saline, did not induce any measurable c-*fos* autoradiographic signal in striatum and in the frontal cortex.

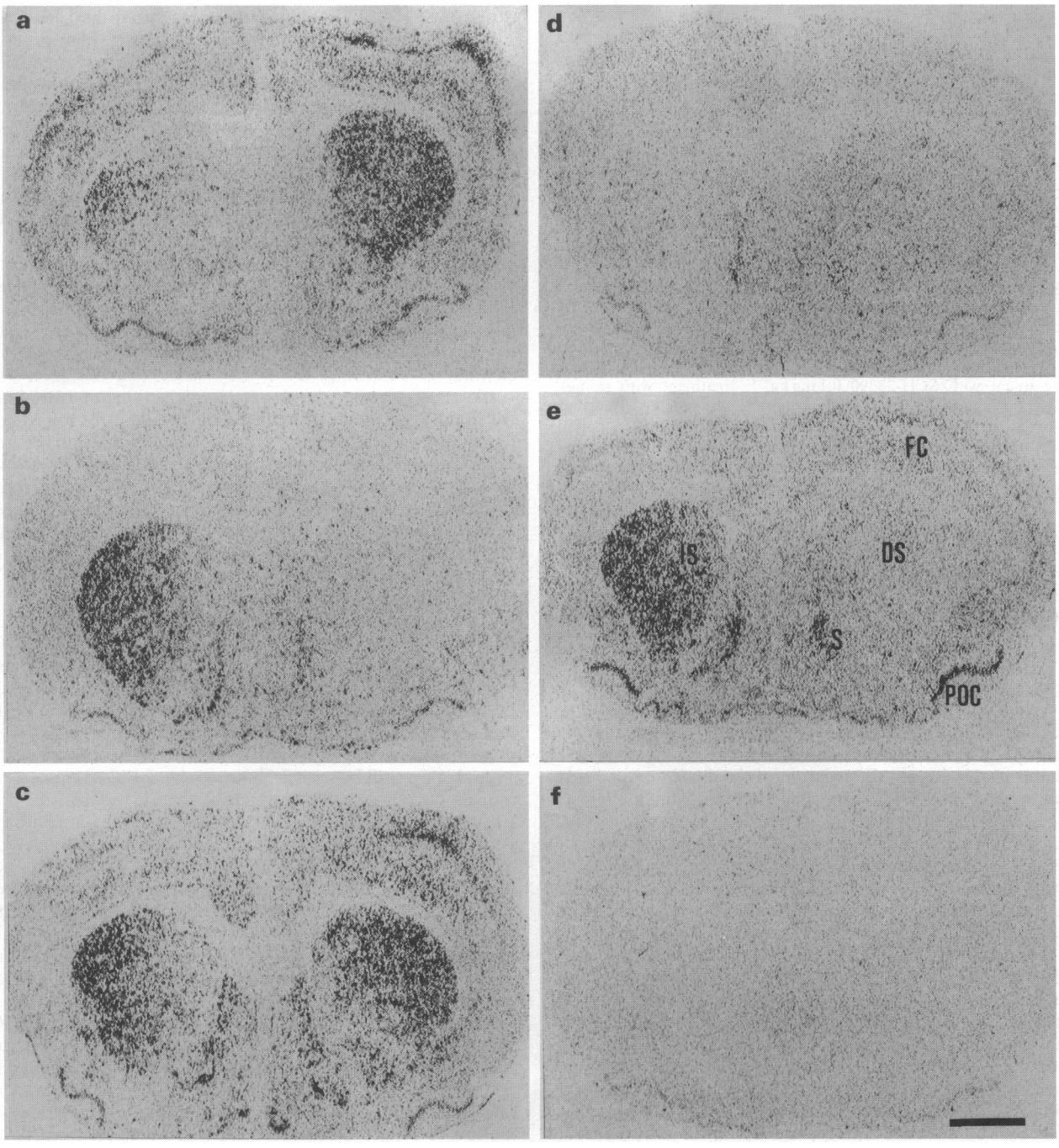

Figure 9 *In situ* hybridization autoradiograms of coronal sections of brains of 6-OHDA-lesioned rats, showing the effect of pretreatments with SCH-23390, on (a) LEK-8829-induced (Sal + LEK) or (b) haloperidol-induced (Sal + Hal) expression of c-*fos* mRNA. Pretreatment with SCH-23390 prevented the induction of c-*fos* mRNA by LEK-8829 (d, SCH + LEK), but not the c-*fos* mRNA expression induced by haloperidol (e, SCH + Hal). The induction of c-*fos* mRNA after pretreatment with haloperidol followed by LEK-8829 (c, Hal + LEK) shows regional summation of autoradiographic signal. The pretreatment with SCH-23390 followed by saline (f, SCH + Sal) did not induce c-*fos* mRNA expression. The rats were killed 30 min after the treatment with drugs. IS - intact striatum, DS - dopamine deafferentated striatum, FC - frontal cortex, S - septal nuclei, POC - primary olfactory cortex. Pretreatments (20 min) and treatments: Sal + LEK (saline, LEK-8829 0.25 mg kg^{-1}); SCH + LEK (SCH-23390 0.1 mg kg^{-1}, LEK-8829 0.25 mg kg^{-1}); Sal + Hal (saline, haloperidol 5 mg kg^{-1}); SCH + Hal (SCH-23390 0.1 mg kg^{-1}, haloperidol 5 mg kg^{-1}); Hal + LEK (haloperidol 5 mg kg^{-1}, LEK-8829 0.25 mg kg^{-1}); SCH + Sal (SCH-2339 0.1 mg kg^{-1}, saline). Bar = 2 mm.

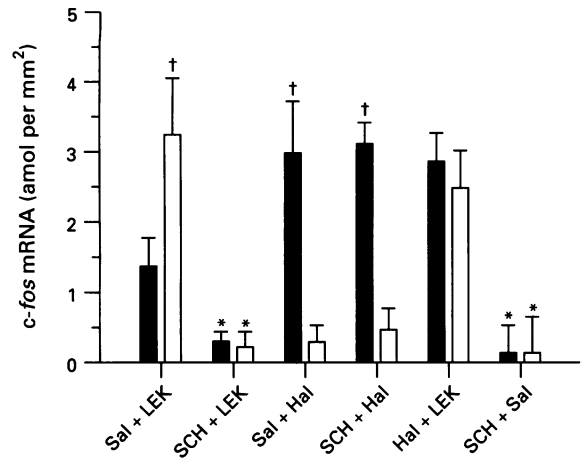

Figure 10 Striatal levels of c-*fos* mRNA in coronal slices of 6-OHDA-lesioned rats after treatment with drugs. Columns represent mean c-*fos* mRNA concentrations; vertical lines show s.d., $n=3$. Open columns, dopamine deafferentated striatum; solid columns, intact striatum. Sal + LEK (pretreatment with saline, treatment with LEK-8829 0.25 mg kg^{-1}); SCH + LEK (pretreatment with SCH-23390 0.1 mg kg^{-1}, treatment with LEK-8829 0.25 mg kg^{-1}); Sal + Hal (pretreatment with saline, treatment with haloperidol 5 mg kg^{-1}); SCH + Hal (pretreatment with SCH-23390 0.1 mg kg^{-1}, treatment with haloperidol 5 mg kg^{-1}); Hal + LEK (pretreatment with haloperidol 5 mg kg^{-1}, treatment with LEK-8829 0.25 mg kg^{-1}); SCH + Sal (pretreatment with SCH-23390 0.1 mg kg^{-1}, treatment with saline). Statistical evaluation: †significantly higher c-*fos* mRNA levels as compared to the contralateral striatum ($n=3$, $P<0.01$, paired Student's t test); *significantly lower c-*fos* mRNA levels as compared to the LEK-8829-induced c-*fos* mRNA levels in the intact/lesioned striatum in Sal + LEK rats ($n=3$, $P<0.01$, unpaired Student's t test).

Low expression of c-*fos* mRNA was found in the primary olfactory cortex in both hemispheres (Figures 9 and 10, SCH + Sal).

Discussion

In the 6-OHDA model, after degeneration of dopaminergic nigrostriatal neurones, supersensitivity of dopaminoceptive mechanisms develops in the striatum of the deafferentated side (Ungerstedt, 1971a). Application of direct dopamine agonists induces contralateral turning behaviour because of preponderant stimulation of supersensitive receptors of the deafferentated side. Application of indirectly acting dopamine agonists (for example amphetamine) induces ipsilateral turning by increasing the synaptic concentration of dopamine in the striatum of the intact side (Ungerstedt, 1971a,b). The ipsilateral turning induced by indirectly acting dopamine agonists can be blocked by antagonists of either D_1 or D_2 receptors. The uncoupling of functional co-operativity between D_1 and D_2 receptors is one of the facets of dopaminergic supersensitivity of the dopamine deafferentated striatum (Hu *et al.*, 1990). The functional uncoupling of dopamine receptors in the dopamine depleted striatum is inferred from the fact that contralateral turning behaviour, induced by mixed D_1:D_2 agonists, cannot be blocked by selective antagonists of D_1 or D_2 receptors (Sonsalla *et al.*, 1988). On the other hand, the contralateral turning behaviour that can be induced by selective stimulation of either D_1 or D_2 receptors, is sensitive only to inhibition by the antagonists of the respective dopamine receptor subtype (Sonsalla *et al.*, 1988; Waddington & Daly, 1993).

In our experimental animals, the injection of 6-OHDA in the median forebrain bundle induced retrograde degeneration of dopamine containing neurones in both the substantia nigra and ventral tegmental area, as shown by the complete loss of TH mRNA signal in the substantia nigra/ventral tegmental area on the 6-OHDA lesioned side. In 6-OHDA-lesioned rats LEK-8829 induced a prolonged (up to 8 h) and intense contralateral turning. The intense response to the drug developed after previous sensitization (priming) with apomorphine. The sensitization of the turning response induced by apomorphine has been described previously (Morelli *et al.*, 1989; Klug & Norman, 1993). The response to LEK-8829 stabilized after repeated weakly applications, both in duration and intensity (rotational speed) of the turning response. The selective antagonists of D_1 receptors, SCH-23390, D_2 receptors, haloperidol, and 5-HT_{1A} receptors, pindolol, were used to demonstrate the mechanism of action of LEK-8829. Usual pharmacological doses of SCH-23390 significantly antagonized the LEK-8829-induced turning behaviour. Pretreatment with high doses of haloperidol did not inhibit turning behaviour to a significant degree, although it affected the profile of the turning response. The effect of pretreatment with pindolol was checked because *in vitro* experiments showed a high affinity of LEK-8829 for 5-HT_{1A} receptors. Namely, partial agonism at 5-HT_{1A} receptors could contribute to the contralateral rotations induced by LEK-8829. Pretreatment with pindolol did not significantly affect the total number of turns or the average turning speed, although the latency to the onset of turning was slightly increased. The dose-dependent inhibition of LEK-8829-induced turning behaviour by SCH-23390, and the lack of significant inhibition of turning after the pretreatment with high doses of haloperidol or pindolol indicate that the actions of LEK-8829 in dopamine deafferentated striatum are mediated by D_1 receptors.

To clarify further the dopaminergic actions of LEK-8829, we studied the effects of the drug on striatal c-*fos* mRNA expression. In a time course study on intact animals, in separate experiments not shown in this paper, we found that LEK-8829 induced a regionally specific pattern of c-*fos* mRNA expression with peak c-*fos* mRNA levels at 30 min, declining to the basal levels thereafter. Similar time-courses of striatal c-*fos* mRNA expression, induced by haloperidol, have been described by other authors (Nguyen *et al.*, 1992; Konradi *et al.*, 1993). The animals in our experiments were, therefore, killed 30 min after the application of c-*fos* mRNA-inducing drugs. Using the 6-OHDA model, we tested the possibility that LEK-8829 at the doses used in our experiments, induced striatal c-*fos* mRNA expression via D_1 receptor stimulation or/and via blockade of D_2 receptors. We therefore, compared the effect of the D_1 receptor antagonist SCH-23390 on striatal c-*fos* mRNA expression induced by either LEK-8829 or by haloperidol. The striatal c-*fos* mRNA levels induced by LEK-8829 were increased both in the dopamine deafferentated and in the contralateral intact striatum. On the other hand, the induction of c-*fos* mRNA transcripts was completely blocked on both sides by pretreatment with SCH-23390. Previous experiments performed on normal rats have revealed antagonism of the effects of LEK-8829 in several D_2 receptor-mediated behavioural paradigms (Krisch *et al.*, 1994). The absence of induction by LEK-8829 of c-*fos* mRNA expression in the dopamine-preserved striatum of unilaterally 6-OHDA-lesioned animals pretreated with SCH-23390, was therefore unexpected. In agreement with the experiments of Dragunow *et al.* (1990), haloperidol-induced expression of c-*fos* mRNA in striatum was limited only to the intact striatum and was not affected by pretreatment with SCH-23390. It could be speculated, therefore, that the dose of LEK-8829 used in the present study was too low to induce D_2 receptor inhibition-mediated expression of c-*fos* mRNA in the intact striatum. We have recently found, in normal rats, that pretreatment with SCH-23390 (1 mg kg^{-1}) completely prevents the induction of c-*fos* mRNA expression induced by 0.25 mg kg^{-1} LEK-8829 but not by 3 mg kg^{-1} LEK-8829 (unpublished data). Therefore, it seems possible that the D_1 receptor stimulation-linked c-*fos* mRNA expression induced by LEK-8829 is already evident at lower doses of the drug, compared to c-*fos* mRNA expression induced by the D_2 receptor-blocking dose. These results apparently disagree with the receptor affinities determined *in vitro* (Krisch *et al.*,

1994), showing higher affinity of LEK-8829 for D_2 as compared to D_1 receptors. The probable explanation for the differences between the *in vitro* affinities and *in vivo* effective doses of the drug may be in the presence of a high proportion of spare D_1 receptors in the striatum (Battaglia 1986; Watts *et al.*, 1995). Further experiments are needed to clarify this issue.

Our results indicate that in the 6-OHDA model, LEK-8829 acts as D_1 receptor agonist both in normally innervated and in dopamine deafferentated D_1 receptors. The striatal c-*fos* mRNA levels induced by LEK-8829 were higher on the lesioned side. This could be the result of the development of postsynaptic supersensitive D_1 receptor-linked mechanisms on the dopamine-depleted side. The possible concomitant antagonism of LEK-8829 at D_2 receptors was, at the doses used, not shown by additional expression of c-*fos* mRNA and was not able to prevent the contralateral turning behaviour induced by the agonist activity of the same drug. It has been shown by other investigators that D_2 receptor inhibition with selective D_2 receptor antagonists cannot prevent the D_1 receptor-mediated contralateral turning behaviour in the 6-OHDA model (Sonsalla *et al.*, 1988). Moreover, FCE-23884, another ergoline derivative with mixed D_2 antagonist/D_1 agonist properties comparable to LEK-8829, also induces a dose-dependent contralateral behaviour in 6-OHDA model (Buonamici *et al.*, 1991).

The expression of c-*fos* mRNA was also found in extrastriatal regions. The c-*fos* mRNA expression seems to be intrinsic to the primary olfactory cortex and seems to be additionally increased under stimulation by LEK-8829. LEK-8829 induced marked elevation of c-*fos* mRNA in the right frontal cortical region, especially in the outer cortical laminae. This effect of LEK-8829 was inhibited by SCH-23390 and may be linked to dopamine D_1 receptor stimulation by the drug. The injection of 6-OHDA in the median forebrain bundle also induced degeneration of dopamine neurones in the ventral tegmental area, the origin of mesocortical dopaminergic projections. The dopaminergic cortical deafferentation may be the cause of D_1 receptor-linked induction of c-*fos* mRNA in the cortical region of the lesioned side. The induction of c-*fos* mRNA in the intact striatum by haloperidol was not associated with an increase of c-*fos* mRNA in the frontal cortex. A study of the effects of LEK-8829 on D_1 receptor linked expression of c-*fos* mRNA in specific regions (striatum, nucl. accumbens, septum and medial frontal cortex) in rats with intact striata is in progress.

Besides dopamine receptor activity, LEK-8829 exhibits a blocking action on 5-HT_2 receptors (antagonism of 5-hydroxytryptophan (5-HTP)-induced head twitches; Krisch *et al.*, 1994) and on 5-HT_{1A} receptors (antagonism of 8-OH-DPAT-induced spontaneous tail flicks in rats; Krisch, personal communication). The experiments with pindolol showed that LEK-8829-induced turning behaviour was not mediated via stimulation of 5-HT_{1A} receptors. The antagonistic activity of the drug on 5-HT receptors could even reduce D_1 receptor-mediated expression of c-*fos* mRNA (Bhat *et al.*, 1992; Bhat & Baraban, 1993).

Stimulation of dopamine D_1 receptors in frontal cortical areas in combination with D_2 and 5-HT receptor antagonism may be promising for the balanced treatment of both positive and negative symptoms of schizophrenia (Kahn & Davis, 1995). Other ergoline derivatives similarly exhibiting D_1 agonistic action together with potent antagonism on other receptor types (D_2 and 5-HT receptors) are compounds CK-204-933 (Markstein *et al.*, 1987) and FCE-23884 (Buonamici *et al.*, 1991; Carfagna *et al.*, 1991; Buonamici *et al.*, 1992). More selective D_1 agonistic action was found with the benzergoline derivative CY-208-243 (Temlett *et al.*, 1989; Markstein *et al.*, 1992). This drug was developed for the treatment of parkinsonism (c.f. Waddington, 1993). LEK-8829, with its mixed D_2 and 5-HT_2 antagonist (Krisch *et al.*, 1994) and D_1 agonist (this investigation) profile seems not to be appropriate for the treatment of uncomplicated parkinsonism. However the compound could be, due to its neuroleptic properties combined with D_1 agonistic activity, potentially useful in the treatment of patients developing psychotic symptoms induced by anti-parkinsonian medication (Greene *et al.*, 1993). It may also prove useful as an experimental tool for the study of D_1:D_2 receptor interactions.

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