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Modulation of Neuroleptic Activity of 9,10-Didehydro-*N*-methyl-(2-propynyl)-6-methyl-8-aminomethylergoline Bimaleinate (LEK-8829) by D1 Intrinsic Activity in Hemi-Parkinsonian Rats

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ABSTRACT

Parkinsonism, a common unwanted side effect of typical antipsychotic (neuroleptic) drugs, is induced by the blockade of striatal dopamine D2 receptors. In rats with hemi-parkinsonism induced by unilateral lesion of dopaminergic nigrostriatal neurons with 6-hydroxydopamine, D2 antagonists inhibit contralateral turning induced by D2 agonists and augment the levels of neurotensin mRNA in dopaminergically intact striatum. By contrast, D1 agonists induce contralateral turning and augment neurotensin mRNA levels in dopamine-depleted striatum. These effects could be inhibited by D1 but not by D2 antagonists. Here we used a hemi-parkinsonian model to investigate the effects of putative D1 agonist/D2 antagonist LEK-8829 (9,10-didehydro-*N*-methyl-(2-propynyl)-6-methyl-8-aminomethylergoline bimaleinate), an experimental antipsychotic, on turning behavior and the expression of striatal neurotensin, preprotachykinin and neurotransmitter-induced early gene protein 4 (ania-4) mRNAs. We found that LEK-8829 inhibited contralateral turning induced by D2 agonist quinpirole, but only if the rats were cotreated with D1 antagonist SCH-23390. In situ hybridization showed that LEK-8829 induced the expression of neurotensin and ania-4 mRNAs in dopamine-intact striatum that could be completely blocked only by the combined treatment with SCH-23390 and quinpirole. In addition, LEK-8829 augmented the expression of neurotensin, preprotachykinin and ania-4 mRNAs in dopamine-depleted striatum that could be completely blocked by SCH-23390. This study clearly demonstrates that in hemi-parkinsonian rats D1 agonistic activity of LEK-8829 confers its anti-parkinsonian drug-like properties and modulates its neuroleptic drug-like properties, which are dependent on the blockade of dopamine D2 receptors. These findings imply that atypical antipsychotics with D1 intrinsic activity might have a reduced propensity for the induction of extrapyramidal syndrome.

The blockade of dopamine D2 receptors by typical antipsychotics (neuroleptics) often provokes unwanted extrapyramidal syndrome (EPS), characterized by parkinsonism, akathisia, catalepsy, and, after long-term treatment, tardive dyskinesia (Deniker, 1990). Fortunately, atypical antipsychotic drugs, such as clozapine, were developed that have a lower tendency for the induction of EPS. Atypical antipsychotics are characterized by high-affinity ratio between serotonin 5-HT2 and dopamine D2 receptors (Meltzer et al., 1989). Their low propensity for the induction of EPS is thought to depend on their ability to preferentially inhibit mesolimbic dopaminergic system as opposed to neuroleptic drugs that effectively inhibit both mesolimbic and mesostriatal dopaminergic systems (Scatton and Sanger, 2000).

The receptor binding profile of 9,10-didehydro-*N*-methyl-(2-propynyl)-6-methyl-8-aminomethylergoline bimaleinate (LEK-8829) was determined in vitro by displacement of appropriate radioligands in preparations of rat brain membranes and of cells expressing recombinant subtypes of human dopamine and serotonin receptors. LEK-8829 is a nonselective compound that binds with high affinity to dopamine D2 and D3 receptors and serotonin 5-HT1A, -2A, -6, and -7 receptors. It also has moderate to low affinity for serotonin 5-HT2C, dopamine D1, D5, and D4 receptors, α1- and α2-adrenergic receptors and sigma receptors. The pK_i ratio 5HT2A/D2 of LEK-8829 is 1.11, that is closer to the pK_i ratio 5HT2A/D2 of atypical antipsychotic clozapine than to the pK_i ratio 5HT2A/D2 of typical antipsychotic haloperidol. According to Meltzer's classification, LEK-8829 was thus classified among atypical antipsychotics (Krisch et al., 1996).

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ABBREVIATIONS: EPS, extrapyramidal syndrome; 5-HT, 5-hydroxytryptamine; LEK-8829, 9,10-didehydro-*N*-methyl-(2-propynyl)-6-methyl-8-aminomethylergoline bimaleinate; 6-OHDA, 6-hydroxydopamine; PPT, preprotachykinin; NT, neurotensin; ania-4, activity and neurotransmitter-induced early gene protein 4; quinpirole hydrochloride, *trans*-(−)-4a*R*-4a,5,6,7,8,8a,9-octahydro-5-propyl-1*H*-pyrazolo[3,4-g]-quinoline hydrochloride; SCH-23390, *R*(+)7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrochloride; TH, tyrosine hydroxylase; SSC, standard saline citrate; ROD, relative optical density; ROI, region of interest; ANOVA, analysis of variance.

At high concentrations (100 μM), LEK-8829 has stimulatory activity on dopamine D1/D5 receptor-mediated cAMP accumulation in C6D1 cells. LEK-8829 inhibited quinpirole-induced mitogenesis in CHOp-D2 and CHOp-D3 cells (I. Krisch, personal communication). Agonistic activity of LEK-8829 on dopamine D1 receptors and antagonistic activity on dopamine D2 receptors were demonstrated also in vivo in rats with unilateral striatal lesions with ibotenic acid (Šprah et al., 1999).

The potential of LEK-8829 for the induction of catalepsy and for the blockade of behaviors induced by dopaminergic agonists has been described as relatively low (Krisch et al., 1994). This indicates that LEK-8829 might have a low propensity for the induction of EPS. Clozapine, a model atypical antipsychotic that is known for its low propensity for extrapyramidal effects, also seems to have intrinsic activity at dopamine D1 receptors (Ahlenius, 1999). It may be speculated, therefore, that the D1 agonistic activity could play a pivotal role in regard to the effects of LEK-8829.

In rats that are rendered hemi-parkinsonian by unilateral lesions of nigrostriatal neurons with 6-hydroxydopamine (6-OHDA model), D1 agonists trigger contralateral turning and augment the expression of striatal neuropeptide mRNAs, such as preprotachykinin (PPT), neurotensin (NT), and neurotransmitter-induced early gene protein 4 (ania-4), preferentially in dopamine-depleted striatum (Sonsalla et al., 1988; Gerfen et al., 1990; Berke et al., 1998; Hanson and Keefe, 1999). By contrast, D2 antagonists block contralateral turning induced by D2 agonists and augment the expression of NT and ania-4 mRNAs, only within the intact striatum (Sonsalla et al., 1988; Berke et al., 1998; Hanson and Keefe, 1999).

In 6-OHDA model, LEK-8829 induced contralateral turning and c-*fos* mRNA in dopamine depleted-striatum that could be blocked by D1 antagonist SCH-23390, but not by D2 antagonist haloperidol (Živin et al., 1996). These results indicated D1 agonistic activity of LEK-8829. On the other hand, in combination with SCH-23390, LEK-8829 antagonized the effects of D2 agonist bromocriptine, indicating D2 antagonistic activity of LEK-8829 (Živin et al., 1998).

We decided to monitor the effects of LEK-8829 on the expression of PPT, NT, and ania-4 mRNAs in 6-OHDA model because these mRNAs are differentially modulated by D1 agonists and D2 antagonists in dopamine-intact and dopamine-depleted striatum. PPT mRNA is expressed predominantly within striatonigral neurons (Gerfen et al., 1990), whereas NT and ania-4 mRNAs are probably expressed in striatonigral and in striatopallidal neurons (Berke et al., 1998; Hanson and Keefe, 1999). In the intact striatum of 6-OHDA animals, D2 receptor-bearing striatopallidal and D1 receptor-bearing striatonigral output pathways are inhibited and stimulated by dopamine, respectively. On the lesioned side, because of the lack of endogenous dopamine, the striatopallidal pathway is maximally facilitated and striatonigral pathway is maximally inhibited (Obeso et al., 2000). In the present study, we therefore hypothesized that LEK-8829 could differentially modulate the motor outflow and striatal expression of NT, PPT, and ania-4 mRNAs on the intact and dopamine–depleted side. To evaluate which effects of LEK-8829 could be attributed to the blockade of dopamine D2 and which to the stimulation of dopamine D1 receptors, we pretreated groups of LEK-8829–treated animals by D1 antagonist SCH-23390 and/or by D2/D3 agonist quinpirole.

Materials and Methods

Animals. We used 62 female Wistar rats. The animals were maintained on a 12-h light/dark cycle (lights on, 7:00 AM to 7:00 PM) in a temperature-controlled colony room at 22 to 24°C with free access to rodent pellets and tap water. Groups of four animals were housed in standard plastic cages with sawdust cover on the floor throughout the experiment. They were handled according to the NIH Guide for the Care and Use of Laboratory Animals.

Drugs. The following drugs were used: apomorphine hydrochloride (Sigma, St. Louis, MO) and *trans*-(−)-4a*R*-4a,5,6,7,8,8a,9-octahydro-5-propyl-1*H*-pyrazolo[3,4-g]-quinoline hydrochloride (quinpirole hydrochloride; Sigma/RBI, Natick, MA) were dissolved in 0.9% saline containing 0.02% ascorbic acid; LEK-8829 (LEK, Ljubljana, Slovenia) was dissolved in 0.9% saline; *R*(+)7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrochloride (SCH-23390; RBI) was dissolved in dimethyl sulfoxide; the final solution was made up with 0.9% saline and dimethyl sulfoxide (2:1). The doses given refer to the form indicated above, except for LEK-8829, which was calculated as the base. The drugs were administered in a volume of 2 ml/kg s.c., except for quinpirole, which was injected i.p.

6-Hydroxydopamine Lesions of the Nigrostriatal Pathway. The stereotaxic lesions were performed on experimentally naive female rats, weighing between 150 and 200 g. The animals were anesthetized with an i.p. injection of 2% xylazine hydrochloride (8 mg/kg; Rompun; Bayer, Leverkusen, Germany), ketamine hydrochloride (60 mg/kg; Ketanest; Parke Davis, Wien, Austria), atropine (0.6 mg/kg; Belupo, Koprivnica, Croatia) and placed in a stereotaxic frame (TrentWells, South Gate, CA). 6-Hydroxydopamine hydrobromide (8 μg of free base dissolved in 0.9% saline containing 0.02% ascorbic acid; RBI) was infused at a rate of 1 μl/min over 4 min into the right medial forebrain bundle at the following coordinates: anterior, 3 mm; lateral, 1.2 mm; and ventral, 7.3 mm; from lambda, midline, and the surface of the dura, respectively (stereotaxic coordinates; Paxinos and Watson, 1998). The incisor bar was set 2.3 mm below the interaural line. The infusion was delivered via 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 4 weeks 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 Lablinc automated rotometer system (Colbourn Instruments, Allentown, PA) designed for the electromechanical recording (Ungerstedt and Arbuthnott, 1970) of turning behavior of eight animals simultaneously. The data files of the turning profiles of each animal (i.e., the full left/right turns per minute) recorded by the L2T2S data acquisition software (Colbourn Instruments) were graphically represented and analyzed using standard Lotus 1–2-3 spreadsheet (Lotus Software, Cambridge, MA), running on a PC.

Apomorphine Test. To determine the development of nigrostriatal degeneration and to stabilize the turning response, the 6-hydroxydopamine–lesioned animals were primed to the stimulation of dopamine D1 and D2 receptors by the treatment with apomorphine hydrochloride (0.05 mg/kg) in the fourth postoperative week. The apomorphine-primed 6-hydroxydopamine–lesioned rats responding with at least 100 contralateral turns during apomorphine test were then randomly divided in experimental groups of four animals for experiments with drugs. The experiments with drugs started 1 week after the priming session with apomorphine.

Drug Treatment. Eight groups of apomorphine-primed 6-hydroxydopamine–lesioned rats were used. Each group was treated in

two experimental sessions, with 1 week of drug-free period between the sessions. In the first experimental session all rats received LEK-8829 (1.7 mg/kg). In the second experimental session, groups received two injections given at 0 min and one injection at 20 min as follows: group 1, Sal/Sal+Sal (n = 7) received three injections of saline; group 2, Sal/Sal+LEK (n = 7) was injected with two injections of saline and LEK-8829 (1.7 mg/kg); group 3, SCH/Sal+LEK (n = 7) was injected with SCH-23390 (1 mg/kg), saline, and LEK-8829 (1.7 mg/kg); group 4, SCH/Sal+Sal (n = 7) was injected with SCH-23390 (1 mg/kg) and two injections of saline; group 5, Sal/Q+LEK (n = 7) was injected with quinpirole (0.25 mg/kg), saline and LEK-8829 (1.7 mg/kg); group 6, Sal/Q+Sal (n = 8) was injected with quinpirole (0.25 mg/kg) and two injections of saline; group 7, SCH/Q+LEK (n = 7) received SCH-23390 (1 mg/kg), quinpirole (0.25 mg/kg), and LEK-8829 (1.7 mg/kg); group 8, SCH/Q+Sal (n = 7) received SCH-23390 (1 mg/kg), quinpirole (0.25 mg/kg), and saline. In addition, two groups of four apomorphine-primed nonlesioned rats were treated in parallel by the same protocols as described for group 1 and group 2. All the animals were killed by decapitation 4 h after the last treatment injection.

Preparation, Fixation, and Storage of Brain Sections. Brains were rapidly removed and quickly frozen on dry ice and stored at −80°C until cryostat sections were cut. Coronal sections (10 μm) were cut through the caudate-putamen and ventral midbrain, then thaw mounted onto glass slides [previously coated with 0.01% solution of poly(L-lysine) in diethyl pyrocarbonate 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 for in situ hybridization histochemistry.

Oligonucleotide Probes. We used oligodeoxyribonucleotide 'antisense' probes (45 bases long) complementary to the rat tyrosine hydroxylase (TH) mRNA (bases encoding 471–515, sequence 5′-AAC CAA ACC AGG GCA CAC AGG GAG AAC CAT GCT GGA CTT CCT AAG-3′), to the rat activity and neurotransmitter-induced early gene protein 4 (ania-4) mRNA (bases encoding 4681–4725, sequence 5′-GGT ACA GCA TTT TCG AGG AGA CTA CAG CAG AGA GGC ATG GAA GCT-3′), to the rat neurotensin/neuromedin N (NT) mRNA (bases encoding 488–532 of exon 4, sequence 5′-GGG TTA ATT GTG TGT GCT CAA TTT TGT TAT AAT CTC TTA TAA TTT-3′), and to the rat mRNA of δ-PPT, a splicing variant of substance P precursor (bases encoding 131–175, sequence 5′-TCG GGC GAT TCT CTG AAG AAG ATG CTC AAA GGG CTC CGG CAT TGC-3′). GenBank accession numbers used to designate the probes were as follows: TH, M23598; ania-4, AF030089; NT, M21187; and PPT, X56306.

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 in the air, and incubated with ^{35}S-labeled 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× Denhardt's solution, 100 μg/ml polyadenylic acid, 10% dextran sulfate, and 40 mM dithiothreitol. The oligodeoxynucleotide probes were labeled at the 3′ end with [^{35}S] dATP (1000–1500 Ci/mmol; PerkinElmer Life Sciences, Boston, MA) and terminal deoxynucleotidyl transferase enzyme (Roche Molecular Biochemcials, 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 1 h in a water bath at 35°C. After the incubation, the labeled probes were purified by a spin column procedure with Sephadex G50. The specific activities of the labeled probes were determined by scintillation counting and ranged from 55 to 150 × 10^3 dpm/μl. Hybridization buffer (100 μl) with labeled probe was applied to each slide, covered with a strip of parafilm and incubated over night (approximately 16 h) at 42°C in a humid chamber to prevent desiccation. For each probe, a few control striatal sections were hybridized in the presence of 100-fold excess of unlabeled probe. The washing was performed for 30 min at room temperature followed by 1-h wash at 55°C in 1× SSC. The sections were then dipped in 0.1× SSC for a few seconds, quickly dehydrated through 50, 70, and 98% ethanol series, dried with a stream of cold air and exposed to X-ray film (Hyperfilm β-max; Amersham Biosciences, Uppsala, Sweden). The autoradiograms were exposed at room temperature for 2 to 3 weeks and developed using standard darkroom techniques.

Image Analysis. The semiquantitative densitometry of hybridization signals of striatal NT, PPT, and ania-4 mRNAs was performed by using MCID, M4 image analyzer (Imaging Research Inc., Canada). The relative optical density (ROD) of hybridization signal of individual mRNAs was determined on dopamine-intact and -depleted sides for each animal by manual outlining (with the help of stereotaxic atlas) of the dorsal striatal region (region of interest; ROI). The measurements were taken only for animals with no detectable TH and NT mRNA signals at the ventral midbrain level on the 6-OHDA–lesioned side. The lower limit of ROD threshold was visually adjusted to the level that eliminated pixels pertaining to corpus callosum (the region with presumably no hybridization signal). The first parameter measured was the ROD of the area covered by the above-threshold pixels within ROI (ROD_{ROI}). Background ROD (i.e., ROD_{bckg} of corpus callosum on the same section) was measured separately and was subtracted from each ROD_{ROI} value. The second parameter measured was the number of the above-threshold pixels within ROI (Total target area; A). The total target area covered by PPT or ania-4 mRNA hybridization signals corresponded to the anatomical outline of dorsal striatum. The total target area of the above-threshold NT mRNA hybridization signal, however, was smaller than the anatomical outline of striatum and varied depending on the treatments, probably because different numbers of striatal neurons were recruited by different treatments. We therefore determined the integrated relative optical density iROD for the NT mRNA hybridization signal ($iROD = (ROD_{ROI} - ROD_{bckg}) \times A$). For each animal, the average intensity of the individual mRNA hybridization signals was finally calculated from measurements performed on three striatal sections.

Statistical Analysis. For each treatment group, the intensity of contralateral turning and of striatal autoradiographic signals was expressed as means ± S.E.M. (n), where n represents the number of animals per treatment group. Statistical significance of the effects of treatments between treatment groups was evaluated by using one-way analysis of variance (ANOVA) followed by Scheffé's Multiple-Comparison Test. An unpaired Student's t test was performed to determine the statistical significance of the differences of the intensity of autoradiographic signals in denervated striatum of saline treated 6-OHDA rats in comparison with the intensity of autoradiographic signals in the striatum of saline treated, nonlesioned rats.

Results

Effect of LEK-8829 on Turning Behavior. After a short latency period of a few minutes, the first treatment with LEK-8829 (1.7 mg/kg) induced intensive, long-lasting contralateral turning behavior of apomorphine-primed 6-OHDA animals (n = 7; total number of turns in first 4 h, 1542 ± 114; maximal frequency of turning, 24 ± 2 turns/min). When the same group of animals received LEK-8829 (1.7 mg/kg) again after a 1-week drug-free interval, the animals displayed a rapid onset of even more vigorous contralateral turning (n = 7; total number of turns in first 4 h, 2402 ± 215; $p < 0.01$, two-tailed Student's t test; maximal frequency of turning, 26 ± 2 turns/min; Fig. 1A, group Sal/Sal+LEK). Contralateral turning was occasionally interrupted by periods of stereotyped behavior characterized by contralateral twisting, compulsive licking, or scratching of the contralateral fore paw with the teeth and/or in the region close to the hind leg.

The interfering behavior apparently reduced the mean rotational speed and the total number of turns performed by the animals. Pretreatment with selective antagonist of dopamine D1 receptors, SCH-23390 (1 mg/kg), which did not induce turning (Fig. 2, group SCH/Sal+Sal), almost completely prevented the induction of turning by LEK-8829 (Figs. 1B and 2, group SCH/Sal+LEK). Treatment with selective agonist of dopamine D2 receptors quinpirole (0.25 mg/kg) that induced a rapid onset of intensive contralateral turning (Figs. 1D and 2, group Sal/Q+Sal) was significantly intensified by the coadministration of SCH-23390 (Figs. 1E and 2, group SCH/Q+Sal). The pretreatment with quinpirole did not significantly affect the LEK-8829–induced turning (Figs. 1F and 2, group Sal/Q+LEK). The administration of LEK-8829, however, abruptly blocked the contralateral turning induced by the combined treatment with quinpirole and SCH-23390 (Figs. 1C and 2, group SCH/Q+LEK).

Effect of Unilateral 6-Hydroxydopamine Lesions on Striatal mRNA Expression. In situ hybridization histochemistry of TH mRNA was performed to select the animals showing a complete degeneration of dopaminergic neurons. 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 effects-of-treatment study on striatal gene expression. The nearly total degeneration of nigrostriatal neurons resulted in a significant increase of NT mRNA signals and a decrease in PPT mRNA signal in the striatum ipsilateral to the lesioned side (Fig. 3). The 6-OHDA lesions did not affect the striatal ania-4 mRNA signal (Fig. 3).

Effect of LEK-8829 on Striatal mRNA Expression. In dopaminergically deafferentated striatum, LEK-8829 (1.7 mg/kg) significantly increased the intensity of NT, PPT, and ania-4 mRNA autoradiographic signals (group Sal/Sal+LEK; Fig. 4). In dopaminergically intact striatum of LEK-8829–treated 6-OHDA animals, we observed significant increase of NT and ania-4 mRNA signals. PPT mRNA signal, however, was decreased in dopamine-intact striatum of animals after the injection of LEK-8829 (group Sal/Sal+LEK; Fig. 4).

Effect of SCH-23390 on LEK-8829–Induced Striatal Gene Expression. In deafferentated striatum, the pretreatment with SCH-23390 (1 mg/kg) completely prevented LEK-8829–mediated changes of PPT, ania-4, and NT mRNA hybridization signals (group SCH/Sal+LEK; Fig. 4). In dopaminergically intact striatum, SCH-23390 only partially inhibited the LEK-8829–induced increase of NT mRNA signal and reversed LEK-8829–mediated decrease of PPT mRNA signal (group SCH/Sal+LEK, Fig. 4). SCH-23390 did not affect LEK-8829–induced increase of ania-4 mRNA hybridization signal.

Effect of Quinpirole on LEK-8829–Induced Striatal Gene Expression. In the deafferented striatum, pretreatment with quinpirole (0.25 mg/kg) increased LEK-8829–elevated PPT mRNA signal, but did not affect hybridization signals of other monitored mRNAs (group Sal+Q/LEK; Fig. 4). In the innervated striatum, pretreatment with quinpirole did not change LEK-8829–mediated effects on the intensity of NT and ania-4 mRNA signals, but prevented LEK-8829–mediated decrease of PPT mRNA signal (group Sal+Q/LEK; Fig. 4).

Effect of Combined Treatment with SCH-23390 and Quinpirole on LEK-8829–Induced Striatal Gene Expression. In the deafferented striatum, pretreatment with SCH-23390 (1 mg/kg) and quinpirole (0.25 mg/kg) inhibited LEK-8829–mediated increase of NT, PPT, and ania-4 mRNA signals (group SCH+Q/LEK; Fig. 4). In the innervated striatum, the combined pretreatment with SCH-23390 and quinpirole synergistically inhibited LEK-8829–induced changes of NT and ania-4 mRNA signals (group SCH+Q/LEK; Fig. 4).

Effect of Treatments with SCH-23390 and/or Quinpirole on Striatal Gene Expression. In the deafferented

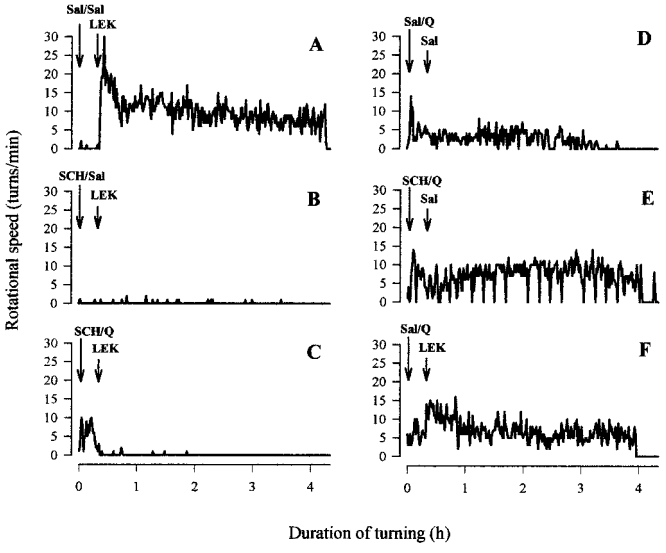

Fig. 1. Recordings of contralateral turning in 6-OHDA–lesioned rats after treatments with different drugs. Each graph is representative recording of turning of one animal. A to F, rotational speed (turns per minute) of rats recorded for 260 min. Animals received first two injections of drugs at 0 min and the last injection at 20 min. Abbreviations and doses of drugs: Sal, saline; LEK, LEK-8829 1.7 mg/kg; SCH, SCH-23390 1 mg/kg; Q, quinpirole 0.25 mg/kg.

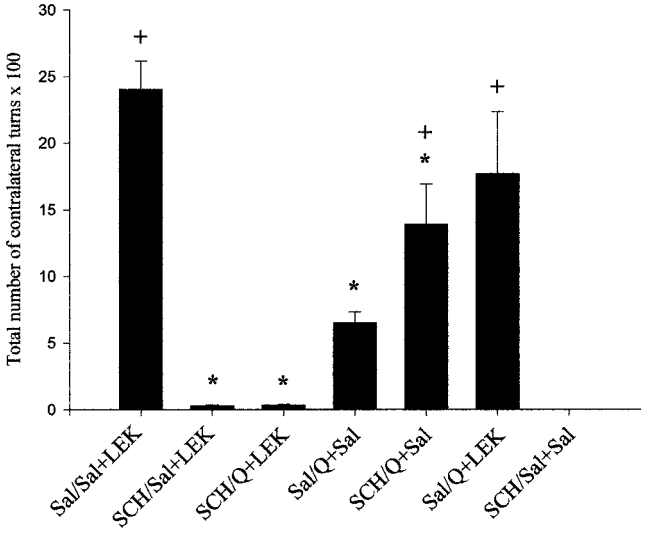

Fig. 2. Total number of turns in first 4 h of 6-OHDA–lesioned rats after pretreatments and treatments with drugs. Columns represent mean cumulative contralateral turns, error bars show S.E.M. Animals received first two injections of drugs at 0 min and the last injection at 20 min. Abbreviations and doses of drugs: Sal, saline; LEK, LEK-8829 1.7 mg/kg; SCH, SCH-23390 1 mg/kg; Q, quinpirole 0.25 mg/kg. Statistical analysis: one-way ANOVA with Scheffé's postcomparative test. (n = 7–8; *, $p < 0.05$ significantly lower total number of turns compared with Sal/Sal+LEK. +, $p < 0.05$ significantly higher total number of turns compared with Sal/Q+Sal).

striatum, neither quinpirole nor SCH-23390 given alone or in combination had any measurable effect on NT or ania-4 mRNA signals, whereas the treatment with quinpirole (0.25 mg/kg) increased PPT mRNA signal (group Sal+Q/Sal; Fig. 5). In innervated striatum, the treatments with quinpirole and/or SCH-23390 did not have any effect on the monitored mRNA signals (Fig. 5).

Effect of LEK-8829 on Regional Distribution of NT mRNA. In saline treated, nonlesioned rats, LEK-8829 (1.7 mg/kg) induced NT mRNA in dorsal striatum, shell, and core of nucleus accumbens, dorsal and ventral parts of lateral septal nuclei, and within olfactory tubercle (Fig. 6, D, E and F). The induction of NT mRNA was more profound in rostral parts of neostriatum and nucleus accumbens than in caudal striatal regions (Fig. 6D).

Discussion

Effects of LEK-8829 on Turning Behavior. All 6-OHDA animals used in this study turned contralaterally after the treatment with low dose of mixed dopamine receptor agonist apomorphine. LEK-8829 induced vigorous, long-lasting contralateral turning in all apomorphine-primed 6-OHDA animals. When the same group of rats received LEK-8829 for the second time after 1 week of drug-free period, the turning became even more vigorous, indicating the development of dopaminergic sensitization.

In 6-OHDA model, contralateral turning could be induced by directly acting dopaminergic agonists that are selective for either pharmacological type of dopamine receptors and inhibited only by antagonists of the same dopamine receptor type that induced the turning (Sonsalla et al., 1988). In contrast, the indirectly acting dopaminergic agonists induce ipsilateral turning by the costimulation of dopamine D1 and D2 receptors on the intact side. Ipsilateral turning is inhibited by the blockade of either type of dopamine receptors (Ungerstedt and Arbuthnott, 1970). The ipsilateral versus contralateral direction of turning probably depends on the preferential induction of motoric outflow from normosensitive and hypersensitive side by indirectly and directly acting dopaminergic agonists, respectively.

Both LEK-8829 and quinpirole induced contralateral turning. LEK-8829–induced turning was inhibited by SCH-23390, implying that it was mediated by hypersensitive dopamine D1 receptors. Contralateral turning induced by quinpirole was sig-

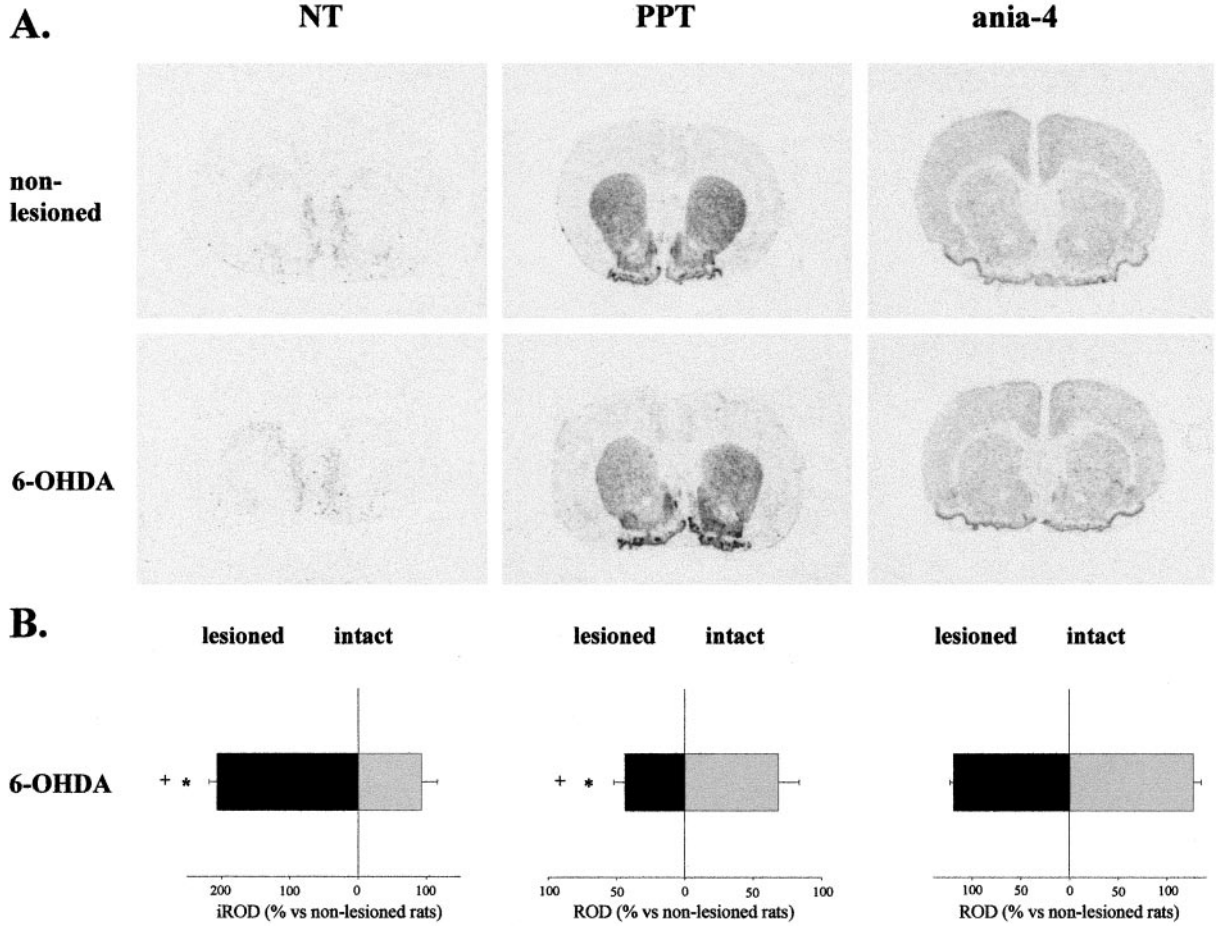

Fig. 3. Effects of unilateral 6-hydroxydopamine lesions on striatal NT, PPT and ania-4 mRNA expression. Normal (nonlesioned) and 6-OHDA rats received one injection of saline at 0 min and two injections of saline at 20 min. All rats were killed 4 h after the last injection. A, representative in situ hybridization images for each type of mRNA signal. Lesioned side is on the left. B, bar charts: average iROD (integrated ROD; iROD = ROD × number of pixels of measured area) for NT, calculated as percentage of average iROD measured from the dorsal striatum of saline treated, nonlesioned rats; average ROD for PPT and ania-4 signals from the dorsal striatum, calculated as percentage of average ROD measured in the striatum of saline treated, nonlesioned rats. Statistical evaluation: *, $p < 0.05$, significantly different mRNA levels compared with the mRNA levels in left striatum of nonlesioned rats (unpaired Student's t test); +, $p < 0.05$ significantly different mRNA levels compared with the mRNA levels in intact striatum of 6-OHDA rats (paired Student's t test). Error bars represent SEM, $n = 7$.

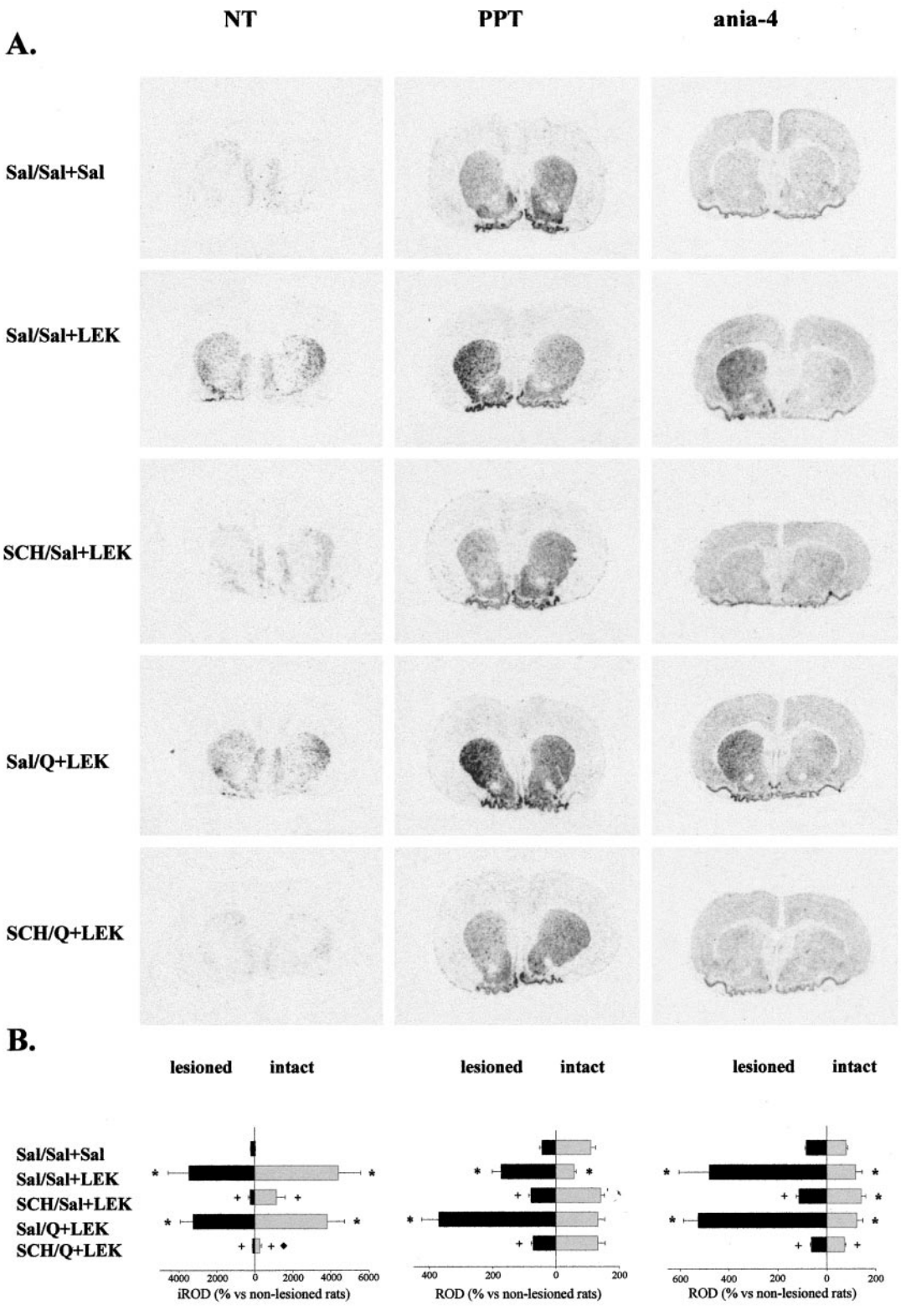

Fig. 4. Effects of treatement with LEK-8829 and pretreatments with SCH-23390 and/or quinpirole on NT, PPT, and ania-4 mRNA expression in striatum of 6-OHDA rats. A, representative in situ hybridization images for each type of mRNA signal. Lesioned side is on the left. B, bar charts: average iROD (iROD = ROD × number of pixels of measured area) for NT, calculated as percentage of average iROD measured from the dorsal striatum of saline treated, nonlesioned rats; average ROD for PPT and ania-4 signals from the dorsal striatum, calculated as percentage of average ROD measured in the striatum of saline-treated, nonlesioned rats. Both deafferented and intact striatum were analyzed, as indicated. Animals received first two injections of drugs at 0 min and the last injection at 20 min. Sal, saline; LEK, LEK-8829 1.7 mg/kg; SCH, SCH-23390 1 mg/kg; Q, quinpirole 0.25 mg/kg. All rats were killed 4 h after the last injection. Statistical analysis: one-way ANOVA and Scheffé's postcomparative test. ANOVA was calculated considering all treatment groups (8): ∗, $p < 0.05$ significantly different mRNA levels compared with the mRNA levels in Sal/Sal+Sal rats; +, $p < 0.05$ significantly lower mRNA levels compared with the mRNA levels in the striatum in Sal/Sal+LEK; ◆, $p < 0.05$ significantly lower mRNA levels compared with the mRNA levels in the intact striatum in SCH/Sal+LEK. Error bars represent S.E.M., for each group $n = 7$.

nificantly amplified by SCH-23390. Selective antagonists of dopamine receptors of the opposite type that induced the turning could amplify the turning (Karlsson et al., 1988). We speculate that the blockade of dopamine D1 receptors by SCH-23390 preferentially inhibits the locomotor outflow on the intact side (by blocking D1 receptor tone exerted by endogenous dopamine) and could thus increase the imbalance of locomotor outflow between dopaminergically intact and deafferented side (caused by the preferential stimulation of hypersensitive dopamine D2 receptors on the lesioned side by quinpirole). By analogy, by

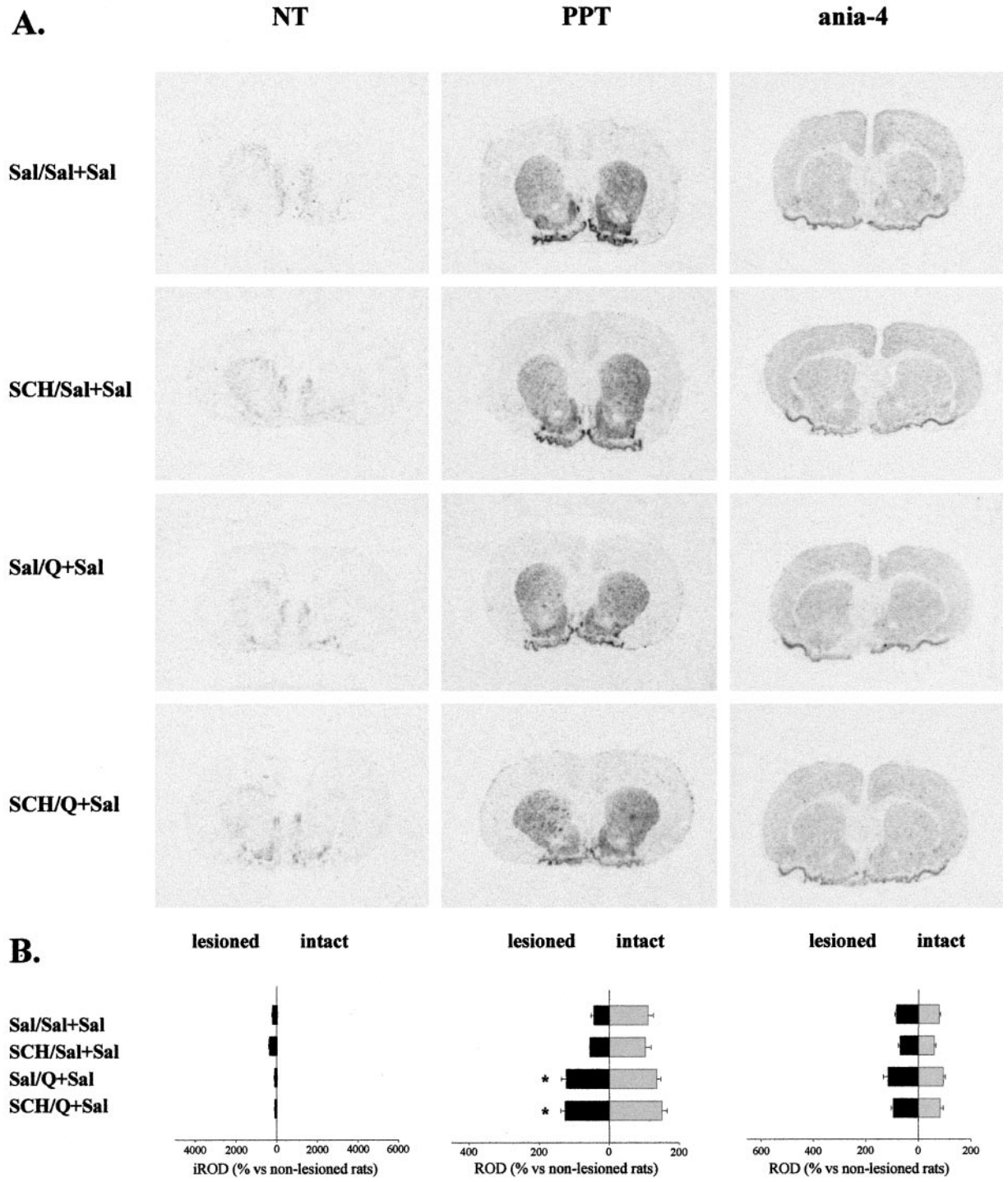

Fig. 5. Effect of combined and separate treatments with SCH-23390 and quinpirole on NT, PPT, and ania-4 mRNA expression in striatum of 6-OHDA rats. A, representative in situ hybridization images for each type of mRNA signal. Lesioned side is on the left. B, bar charts: average iROD (iROD = ROD × number of pixels of measured area) for NT, calculated as percentage of average iROD measured from the dorsal striatum of saline treated, nonlesioned rats; average ROD for PPT and ania-4 signals from the dorsal striatum, calculated as percentage of average ROD measured from the striatum of saline treated, nonlesioned rats. Both deafferented and intact striatum were analyzed, as indicated. Animals received first two injections of drugs at 0 min and the last injection at 20 min. Sal, saline; SCH, SCH-23390 1 mg/kg; Q, quinpirole 0.25 mg/kg. All rats were killed 4 h after the last injection. The statistical analysis used one-way ANOVA and Scheffé's postcomparative test. ANOVA was calculated considering all treatment groups (8): *, $p < 0.05$, significantly higher mRNA levels compared with the mRNA levels in the deafferented striatum in Sal/Sal+Sal rats. Error bars represent S.E.M.

blocking D2 receptor tone exerted by endogenous dopamine, LEK-8829 could preferentially inhibit locomotor outflow on the dopaminergically intact side and thus amplify the contralateral turning caused by the stimulation of dopamine D1 receptors on the hypersensitive side. Quinpirole did not significantly affect LEK-8829–induced turning. We therefore assumed that LEK-8829–induced contralateral turning in quinpirole-pretreated animals was driven only by the stimulation of hypersensitive dopamine D1 receptors. To confirm the hypothesis that LEK-8829 is D1 agonist and D2 antagonist, we pretreated the animals with SCH-23390 and quinpirole followed by administration of LEK-8829. We found that LEK-8829 could abruptly block the turning induced by quinpirole/SCH-23390 pretreatment. We argue that at the doses used in this experimental setting, the striatal concentration of LEK-8829 has been sufficient for the displacement of quinpirole from dopamine D2 receptors but insufficient for the displacement of SCH-23390 from dopamine D1 receptors, so that neither quinpirole nor LEK-8829 could mediate the turning (Fig. 1C).

Effects of LEK-8829 on Striatal Gene Expression. We found a nearly complete absence of TH and NT mRNA hybridization signals in substantia nigra compacta and in the ventral tegmental area on the lesioned side. This is consistent with unilateral degeneration of dopaminergic nigrostriatal, mesolimbic, and mesocortical neurons in our experimental animals. In the striatum ipsilateral to the lesion, we found increased abundance of striatal NT mRNA, decreased abundance of striatal PPT mRNA, and unchanged abundance of striatal ania-4 mRNA. These changes may be attributed to the striatal adaptations that developed after nearly complete dopaminergic deafferentation (Sivam et al., 1987; Hanson and Keefe, 1999).

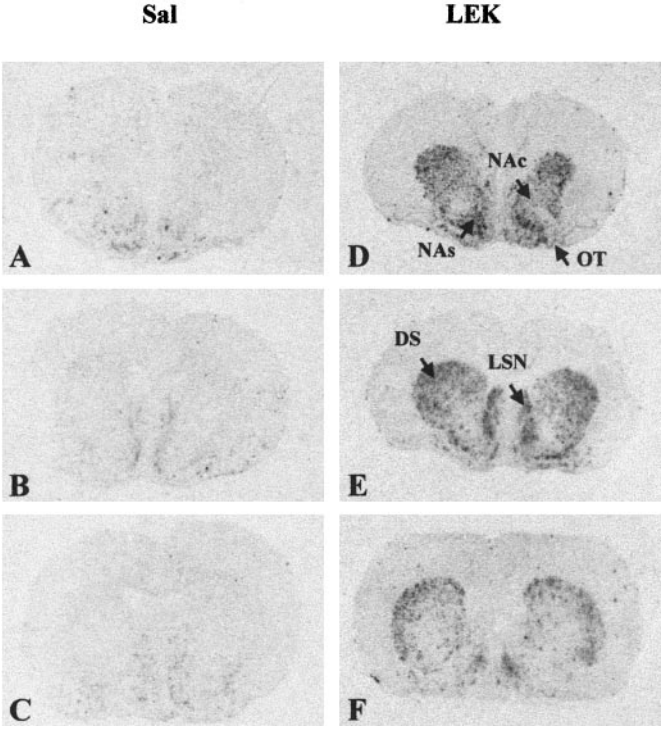

Fig. 6. Distribution of NT mRNA hybridization signal in coronal brain sections of nonlesioned animals 4 h after the treatment with saline (Sal) or LEK-8829 (LEK). Autoradiograms are shown in three different rostrocaudal regions of striatum: A (bregma 2.2 mm), B (bregma 2.0), and C (bregma 0.2 mm) are representative sections from saline-treated animals, whereas D, E, and F are anatomically matched sections from animals treated with LEK-8829 (1.7 mg/kg). DS, dorsal striatum; NAs, shell of nucleus accumbens; Nac, core of nucleus accumbens; LSN, dorsal and ventral parts of lateral septal nuclei; and OT, olfactory tubercle.

Four hours after the injection of LEK-8829, there was up-regulation of PPT, ania-4, and NT mRNAs in the deafferented striatum. SCH-23390, but not quinpirole, inhibited these effects of LEK-8829. This would indicate that in dopamine-depleted striatum, LEK-8829–induced changes were mediated only by the stimulation of dopamine D1 receptors. The interaction of LEK-8829 with both types of dopamine receptors became more evident when we analyzed the induction of NT mRNA by LEK-8829 in dopamine intact striatum. Namely, the induction of NT mRNA was only partially blocked by SCH-23390 and was completely prevented only by combined SCH-23390/quinpirole pretreatment. This suggests that in dopamine-intact striatum, NT mRNA was induced by both the stimulation of dopamine D1 receptors by LEK-8829 and by the blockade of dopamine D2 receptors by LEK-8829. The opposing activity of LEK-8829 at dopamine D1 and D2 receptors within the intact striatum was confirmed also by the effect of LEK-8829 on the expression of ania-4 mRNA, because only the combined quinpirole/SCH-23390 treatment completely blocked the induction of ania-4 mRNA by LEK-8829. Because these effects of LEK-8829 could be completely prevented by quinpirole and/or SCH-23390, it does not seem likely that the inhibition of serotonin receptors by LEK-8829 significantly interfered with the effects of LEK-8829 at dopamine receptors.

LEK-8829 down-regulated striatal PPT mRNA on the dopaminergically intact side and up-regulated it on the lesioned side. These effects were also prevented by SCH-23390/quinpirole cotreatment. It has been shown previously that the antiparkinsonian response could be associated with the correction of the lesion-induced decrease of PPT mRNA. This correction within striatonigral neurons on the dopamine-depleted side could be achieved by selective D1 agonists or selective D2 agonists (Morissette et al., 1999) or by the costimulation of both types of dopamine receptors (Haverstick et al., 1989, Granata et al., 1996). Quinpirole might also induce PPT mRNA directly by stimulation of D3 receptors that are known to be de novo expressed by striatonigral neurons in dopamine-depleted striatum after priming with directly acting dopaminergic agonists (Bordet et al., 2000). Dopamine or serotonin depletion down-regulates striatal PPT mRNA (Walker et al., 1991). It seems that on the dopaminergically intact side, the D2 antagonistic effects of LEK-8829 on PPT mRNA expression prevailed over its D1 agonistic effects, whereas on the dopamine-depleted side, D1 agonistic effects prevailed over D2 antagonistic effects. The ineffectiveness of the blockade of dopamine D2 receptors by LEK-8829 on the lesioned side may be explained by the ongoing inactivity of dopamine D2 receptors due to chronic lack of dopamine. In quinpirole-pretreated animals that were cotreated by saline or by SCH-23390, we observed significant augmentation of striatal PPT mRNA on lesioned side, whereas in quinpirole-pretreated animals that were cotreated with LEK-8829, the augmentation was even more pronounced. It may be speculated that by blocking dopamine D2 receptors, LEK-8829 attenuated further stimulation of PPT mRNA expression induced by quinpirole, whereas LEK-8829 induced even stronger stimulation of PPT mRNA expression because of the stimulation of D1 receptors. By contrast, in LEK-8829–treated animals that received combined pretreatment by quinpirole and SCH-23390, the levels of PPT mRNA were not significantly increased. We

argue that the stimulation by quinpirole was prevented after 20 min by LEK-8829, whereas the stimulation by LEK-8829 in the next 4 h was prevented by SCH-23390. Therefore, PPT mRNA that was induced by quinpirole pretreatment returned to the prestimulation levels.

The induction of NT by neuroleptic drugs has led to the hypothesis that neurotensin may function as an endogenous antipsychotic compound (Nemeroff, 1980). Considerable effort has since been directed toward determining whether NT neurons mediate the effects of antipsychotic drugs. Atypical antipsychotics preferentially induce NT mRNA within nucleus accumbens, whereas typical antipsychotics induce NT mRNA also within corpus striatum (Kinkead et al., 1999). The induction of striatal NT mRNA is thus considered to be an effect of typical antipsychotics (neuroleptics) that induce parkinsonism. LEK-8829 induced NT mRNA in both striatum and nucleus accumbens. LEK-8829 induced NT mRNA within dopamine intact striatum by both D2 antagonistic and D1 agonistic activity. Because D1 agonists oppose, rather than augment, parkinsonism, the induction of striatal NT mRNA by LEK-8829 could not be predictive for the induction of parkinsonism.

Intrinsic activity of LEK-8829 at D1 receptors may be a useful feature in relation to its potential effectiveness against negative symptoms of schizophrenia. D1 agonistic activity of atypical antipsychotic clozapine may be the mechanism that underlies its beneficial effects on negative symptoms, by improving the deranged neural activity of prefrontal cerebral cortex and associated impairments of working memory (Lidow et al., 1998; Ahlenius 1999; Seamans et al., 2001). We propose that D1 agonistic activity of LEK-8829 within basal ganglia could be the mechanism that underlies its relatively low potential for the induction of catalepsy and for the blockade of behaviors mediated by dopaminergic agonists (Krisch et al., 1994). Theoretically, LEK-8829 could also have a low propensity for the development of tardive diskinesia. It was found that up-regulation of dopamine D2 receptors after chronic blockade by neuroleptics that may lead to the development of tardive dyskinesia could be opposed by cotreatment with selective D1 agonists. The cotreatment with D1 agonist might prevent the emergence of EPS without significantly altering the antipsychotic effects of neuroleptics (Braun et al., 1997).

This study confirmed our hypothesis that LEK-8829 is D1 agonist and D2 antagonist. It also showed that the SCH-23390-sensitive induction of contralateral turning and of neuropeptide mRNAs within dopamine-depleted striatum of 6-OHDA rats could be used for the screening of antipsychotics with D1 agonistic activity. Because the induction of striatal neurotensin mRNA could be associated with both antiparkinsonian and antipsychotic drug-like activity of LEK-8829, we propose that atypical antipsychotics with D1 intrinsic activity might have a reduced propensity for the induction of EPS.

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