SL25.1131 [3(S),3a(S)-3-Methoxymethyl-7-[4,4,4-trifluorobutoxy]-3,3a,4,5-tetrahydro-1,3-oxazolo[3,4-a]quinolin-1-one], a New, Reversible, and Mixed Inhibitor of Monoamine Oxidase-A and Monoamine Oxidase-B: Biochemical and Behavioral Profile


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ABSTRACT

SL25.1131 [3(S),3a(S)-3-methoxymethyl-7-[4,4,4-trifluorobutoxy]-3,3a,4,5-tetrahydro-1,3-oxazolo[3,4-a]quinolin-1-one] is a new, nonselective, and reversible monoamine oxidase (MAO) inhibitor, belonging to a oxazoloquinolinone series. In vitro studies showed that SL25.1131 inhibits rat brain MAO-A and MAO-B with IC50 values of 6.7 and 16.8 nM and substrate-dependent Ki values of 3.3 and 4.2 nM, respectively. In ex vivo conditions, the oral administration of SL25.1131 induced a dose-dependent inhibition of MAO-A and MAO-B activities in the rat brain with ED50 values of 0.67 and 0.52 mg/kg, respectively. In the rat brain, duodenum, and liver, the inhibition of MAO-A and MAO-B by SL25.1131 (3.5 mg/kg p.o.) was reversible, and the recovery of MAO-A and MAO-B activities was complete 16 h after administration. SL25.1131 (3.5 mg/kg p.o.) increased tissue levels of dopamine (DA), norepinephrine, and 5-hydroxytryptamine and decreased levels of their deaminated metabolites 3,4-dihydroxyphenylacetic acid, homovanillic acid, and 5-hydroxyindolacetic acid. In mice, SL25.1131 induced a dose-dependent potentiation of 5-hydroxytryptophan-induced tremors and phenylethylamine-induced stereotypies with ED50 values of 0.60 and 2.8 mg/kg p.o., respectively. SL25.1131 was able to reestablish normal striatal dopaminergic tone and locomotor activity in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned mice. In addition, when coadministered with L-DOPA, SL25.1131 increased the available DA in the striatum and the duration of L-DOPA-induced hyperactivity. The duration of the effect of L-DOPA on circling behavior in 6-hydroxydopamine-lesioned rats was also increased. The neurochemical profile of SL25.1131 demonstrates that this compound is a mixed, potent, and reversible MAO-A/B inhibitor in vitro, in vivo, and ex vivo. SL25.1131 has therapeutic potential as a symptomatic treatment during the early phase of Parkinson’s disease and as an adjunct to L-DOPA therapy during the early and late phases of the disease.

Monoamine oxidase (MAO) deaminates monoamine neurotransmitters as well as exogenous amines. Two isoforms of MAO have been described in mammalian tissue, namely, MAO-A and MAO-B, which exhibit different substrate profiles, respond differently to selective inhibitors, and are present in different cellular and subcellular locations. MAO-A preferentially deaminates 5-hydroxytryptamine (serotonin) (5-HT) and norepinephrine (NE) and is selectively inhibited by clorgyline (Johnston, 1968), whereas MAO-B is selectively inhibited by L-deprenyl and preferentially deaminates phenylethylamine (PEA) and benzylamine (Knoll and Magyar, 1972). Dopamine (DA) and tyramine (TYR) are metabolized by both enzyme isoforms (for review, see Strolin-Benedetti and Dostert, 1985).

ABBREVIATIONS: MAO, monoamine oxidase; 5-HT, 5-hydroxytryptamine (serotonin); NE, norepinephrine; PEA, phenylethylamine; DA, dopamine; TYR, tyramine; PD, Parkinson’s disease; L-5-HTP, L-5-hydroxytryptophan; MPTP, 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; 3-MT, 3-methoxytyramine; NMN, normetanephrine; 5-HIAA, 5-hydroxyindolacetic acid; DHBA, 3,4-dihydroxybenzylamine; 6-OHDA, 6-hydroxydopamine; Kapp; substrate-dependent Ki; MAOI, monoamine oxidase inhibitor; SBP, systolic blood pressure; COMT, catechol-O-methyl-transferase; SL25.1131, 3(S),3a(S)-3-methoxymethyl-7-[4,4,4-trifluorobutoxy][3,3a,4,5-tetrahydro-1,3-oxazolo[3,4-a]quinolin-1-one; BW137OU87, 1-ethylphenoxathiin-10,10-dioxide; GBR12935, 1-[2-(disphenyl-methoxy)ethyl]-4-(3-phenylpropyl) piperazine; Ro41-1049, N-(2-aminoethyl)-5-(3-fluorophenyl) thiazole-4-carboxamide hydrochloride; Ro19-6327, lazabemide.
The first generation of irreversible and nonselective MAO inhibitors displayed antidepressant activity but induced serious side effects, including hepatotoxicity, orthostatic hypotension, and most importantly the “cheese effect” characterized by an acute hypertensive crisis when given in combination with foods containing TYR (Bieck and Antonin, 1989). Because both isoforms of MAO metabolize TYR and are present in the intestine, it was assumed that after selective MAO-A inhibition, TYR could be deaminated by MAO-B. However, the next generation of irreversible and selective MAO-A inhibitors (i.e., clorglyline) also induced hypertension when associated with TYR. The lack of tyramine effect with L-deprenyl, a selective and irreversible MAO-B inhibitor, suggests that the tyramine effect with clorglyline may be related to the irreversible nature of the inhibition of MAO-A and not to a nonselective inhibition of both isoforms of MAO. Indeed, the cheese effect, while still present, is less problematic with reversible MAO-A inhibitors such as b-floxatone (Curet et al., 1998) and moclobemide (Da Prada et al., 1989). Whereas reversibility of MAO-A inhibition is associated with a better safety profile, there is no evidence that MAO inhibitors need to be selective for therapeutic effect. Although each MAO isoform shows amine selectivity, each can assume the function of the other when one is inhibited (Butcher et al., 1990). This suggests that nonselective MAO inhibitors might have broader therapeutic effects than selective MAO-A or MAO-B inhibitors. These should be reversible inhibitors to minimize their potential side effects.

Parkinson's disease (PD) is a progressive neurodegenerative disorder of the mesotelencephalic dopaminergic system. The major clinical symptom, i.e., the inability to initiate voluntary movements, can be relieved by L-DOPA (Cotzias et al., 1969). However, a major problem of L-DOPA therapy is the maintenance of a good responsiveness to the drug during long-term therapy (Agid et al., 1995). The success of long-term L-DOPA therapy in PD is compromised by the almost invariable intrusion of dyskinesias during the periods of the day when medication is at its most effective (Agid et al., 1995). After treatment with the drug for more than 5 or 6 years, a majority of patients develop response fluctuations, dyskinesia, or dystonia (Lang and Lozano, 1998a,b). There is therefore a need for novel symptomatic treatments, which could alleviate motor dysfunction in PD at an early stage, and consequently delay the use of L-DOPA. In addition, when associated with L-DOPA treatment in PD at a late stage, this treatment should reduce the fluctuation in the effects of L-DOPA by lengthening its duration of action and therefore diminish its undesirable effects. The irreversible MAO-B inhibitor selegeline (Deprenyl) was developed for the treatment of PD (Birkmayer et al., 1983), and there is some evidence that selegeline monotherapy reduces the “wearing off” akinesia and delays the need for L-DOPA therapy in mild, previously untreated PD by 8 to 9 months (Parkinson Study Group, 1996a). The onset of disability sufficient to require L-DOPA therapy was delayed in patients who received the reversible MAO-B inhibitor lazabemide (Parkinson Study Group, 1996b). However, MAO-A inhibitor can also deaminate dopamine oxidatively, and the reversible MAO-A inhibitor moclobemide has demonstrated therapeutic potential in PD (Sieradzan et al., 1995). Thus, drugs that inhibit both MAO-A and -B may be more efficacious than selective MAO-A or MAO-B inhibitors in PD (Fahn and Chouinard, 1998).

SL25.1131 [3(S),3a(S)-3-methoxymethyl-7-[4,4,4-trifluorobutyl]-3,3a,4,5-tetrahydro-1,3-oxazolo[3,4-a]quinolin-1-one] is an oxazoloquinolinone derivative (Fig. 1) that belongs to a “fourth generation” of MAO inhibitors that combine reversibility and mixed inhibitory activity toward both MAO-A and MAO-B isoenzymes.

The aim of the present study was to establish the biochemical and behavioral profile of SL25.1131 in rodents. In addition, the relative safety of SL25.1131 was compared with that of irreversible mixed MAO-A/B-inhibitors (phenelzine and nialamide) by evaluating the tyramine pressor effect in the rat. Finally, biochemical and behavioral techniques were used to evaluate the therapeutic potential of SL25.1131 in rodent models of PD during the early phase of the lesion and as an adjunct to L-DOPA therapy during the late phase of the lesion.

### Materials and Methods

#### Animals

Studies on MAO activities used male Sprague-Dawley rats (OFA; Ifsa-Credo, L’Arbresle, France) weighing 100 to 200 g. Interaction studies with tyramine were carried on male Sprague-Dawley rats (OFA; Ifsa-Credo) weighing 330 to 664 g on the day of dosing. Male OF1 mice (Ifsa-Credo) weighing 20 to 30 g were used for the potentiation of PEA-induced effects and the potentiation of 1,5-hydroxytryptophan (1,5-HT)-induced effects. The other behavioral studies used male OFA rats and male C57Bl/6 mice (Ifsa-Credo), weighing 180 to 200 g and 16 to 25 g, respectively, at the start of the experiment. The 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) studies used male C57Bl/6 mice (Ifsa-Credo), weighing 20 to 25 g at the start of the experiment. Rats were housed in groups of four in polypropylene cages (37 × 37 × 18 cm), and mice were housed in groups of 8 to 10 in polypropylene cages (22 × 10 × 8 cm). All animals were housed in a controlled environment (light/dark cycles of 12 h with lights on from 7:00 AM to 7:00 PM, temperature of 21 ± 2°C) with food and water ad libitum. This protocol has been approved by the Ethical Committee for Laboratory Animals of Sanofi-Synthelabo Recherche and was carried out in accordance with European Directive 86/609/EEC.

#### Chemicals and Drugs

DA, NE, dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 3-methoxytyramine (3-MT), normetanephrine (NMN), 5-hydroxyindolacetic acid (5-HIAA), 5,4-dihydroxybenzylamine (DHBA), 5-HT, phenelzine sulfate, L-DOPA, benserazide, PEA hydrochloride, tyramine hydrochloride, desmethylimipramine, apomorphine hydrochloride, 6-OHDA, and 2,5-diphenyloxazole were supplied by Sigma-Aldrich (St. Louis, MO). MPTP was obtained from Sigma-Aldrich (St. Louis, MO). L-5-HTP was obtained from Fluka (Mulhouse, France), and ketamine hydrochloride was from Parke Davis (Detroit, MI). Tolcapone was obtained from Roche Diagnostics (Basel, Switzerland). SL25.1131 was synthesized by the Medicinal Chemistry Department (SANOFI-Synthelabo Research, Bagnex, France). 5-hydroxy [side chain-2-14C]-tryptamine creatinine sulfate (specific activity 1.8–2.2 GBq/mmol, [14C]-5-HT) was supplied by Amersham Biosciences UK, Ltd. (Little Chalfont, Buckinghamshire, UK). Phenylethylamine hydrochloride (ethyl-1-14C) (specific activity 1.5–2.2 GBq/mmol, [14C]PEA), was supplied by PerkinElmer Life and Analytical Sciences (Boston, MA). MPTP was obtained from Sigma/
RBI (Natick, MA). Imalgene 500 was obtained from Merial (Lyon, France). Toluene, EDTA (disodium salt of ethylenediaminetetraacetic acid), and ethyl acetate were purchased from Labos (Osie, Paris, France), and Biofluor was from PerkinElmer Life and Analytical Sciences. The analytical-grade buffers NaH2PO4, 2H2O and Na2HPO4, 12H2O, perchloric acid, and sucrose were purchased from Merck (Darmstadt, Germany). All other reagents were standard laboratory reagents of analytical grade.

**Determination of MAO-A and MAO-B Activities**

MAO activities were determined as described previously by Curet et al. (1996).

**In Vitro Experiments.** For IC50 determination, rat forebrains were homogenized in 20 volumes of buffer (0.25 M sucrose, 10 mM sodium phosphate buffer, pH 7.4) at 4°C (final concentration 5 mg of tissue/assay). Briefly, 100 µl of homogenates was preincubated for 20 min at 37°C with various concentrations of inhibitor in a total volume of 400 µl. After this preincubation period, the reaction was started by the addition of [14C]5-HT as the specific MAO-A substrate (final concentration 125 µM, specific activity 1 µCi/µmol) or [14C]PEA as the specific MAO-B substrate (final concentration 8 µM, specific activity 10 µCi/µmol). The final volume of incubation was 500 µl in buffer (0.25 M sucrose, 10 mM sodium phosphate buffer, pH 7.4). The incubation times were 5 min for MAO-A and 1 min for MAO-B. The reaction was stopped by the addition of 200 µl of 4 M HCl. Deaminated metabolites were extracted by vigorous shaking for 10 min in 7 ml of toluene/ethyl acetate (v/v). After extraction, the aqueous phase was frozen with liquid nitrogen, and the organic layer was poured into a scintillation vial to which 0.4 ml/h of 2,5-diphenyloxazole was subsequently added. After 5 min of agitation, radioactivity was measured in a scintillation spectrometer (LS-1801, LS-1701; Beckman Coulter Inc., Fullerton, CA). Blank values were obtained by adding HCl before the substrate. MAO activities were corrected for the efficiencies of extraction of deaminated metabolites into the toluene/ethyl acetate phase (for [14C]PEA, 0.95; for [14C]5-HT, 0.77). IC50 values were calculated from inhibition curves, and IC50 is expressed with various concentrations of inhibitor in a total volume of 400 µl.

For substrate-dependent IC50 (Ki), the reaction was started by the addition of [14C]5-HT as the specific MAO-A substrate (final concentration 125 µM, specific activity 1 µCi/µmol) or [14C]PEA as the specific MAO-B substrate (final concentration 8 µM, specific activity 10 µCi/µmol). The final volume of incubation was 500 µl in buffer (0.25 M sucrose, 10 mM sodium phosphate buffer, pH 7.4). The incubation times were 5 min for MAO-A and 1 min for MAO-B. The reaction was stopped by the addition of 200 µl of 4 M HCl. Deaminated metabolites were extracted by vigorous shaking for 10 min in 7 ml of toluene/ethyl acetate (v/v). After extraction, the aqueous phase was frozen with liquid nitrogen, and the organic layer was poured into a scintillation vial to which 0.4 ml/h of 2,5-diphenyloxazole was subsequently added. After 5 min of agitation, radioactivity was measured in a scintillation spectrometer (LS-1801, LS-1701; Beckman Coulter Inc., Fullerton, CA). Blank values were obtained by adding HCl before the substrate. MAO activities were corrected for the efficiencies of extraction of deaminated metabolites into the toluene/ethyl acetate phase (for [14C]PEA, 0.95; for [14C]5-HT, 0.77). IC50 values were calculated from inhibition curves, and IC50 is expressed with various concentrations of inhibitor in a total volume of 400 µl.

For substrate-dependent IC50 (Ki), MAO-A determination, rat forebrain was homogenized in buffer (0.25 M sucrose, 10 mM sodium phosphate buffer, pH 7.4; 100 mg of tissue/assay). Briefly, 100 µl of homogenates was preincubated for 20 min at 37°C with various concentrations of inhibitor in a total volume of 400 µl. After this preincubation period, the reaction was started by the addition of [14C]5-HT as the specific MAO-A substrate (final concentration 125 µM, specific activity 1 µCi/µmol) or [14C]PEA as the specific MAO-B substrate (final concentration 8 µM, specific activity 10 µCi/µmol). The final volume of incubation was 500 µl in buffer (0.25 M sucrose, 10 mM sodium phosphate buffer, pH 7.4). The incubation times were 5 min for MAO-A and 1 min for MAO-B. The reaction was stopped by the addition of 200 µl of 4 M HCl. Deaminated metabolites were extracted by vigorous shaking for 10 min in 7 ml of toluene/ethyl acetate (v/v). After extraction, the aqueous phase was frozen with liquid nitrogen, and the organic layer was poured into a scintillation vial to which 0.4 ml/h of 2,5-diphenyloxazole was subsequently added. After 5 min of agitation, radioactivity was measured in a scintillation spectrometer (LS-1801, LS-1701; Beckman Coulter Inc., Fullerton, CA). Blank values were obtained by adding HCl before the substrate. MAO activities were corrected for the efficiencies of extraction of deaminated metabolites into the toluene/ethyl acetate phase (for [14C]PEA, 0.95; for [14C]5-HT, 0.77). IC50 values were calculated from inhibition curves, and IC50 is expressed with various concentrations of inhibitor in a total volume of 400 µl.

For substrate-dependent IC50 (Ki), MAO-B determination, rat forebrain was homogenized in buffer (0.25 M sucrose, 10 mM sodium phosphate buffer, pH 7.4; 100 mg of tissue/assay) and preincubated with various concentrations of inhibitor in a total volume of 400 µl. After this preincubation period, the reaction was started by the addition of [14C]5-HT as the specific MAO-A substrate (final concentration 125 µM, specific activity 1 µCi/µmol) or [14C]PEA as the specific MAO-B substrate (final concentration 8 µM, specific activity 10 µCi/µmol). The final volume of incubation was 500 µl in buffer (0.25 M sucrose, 10 mM sodium phosphate buffer, pH 7.4). The incubation times were 5 min for MAO-A and 1 min for MAO-B. The reaction was stopped by the addition of 200 µl of 4 M HCl. Deaminated metabolites were extracted by vigorous shaking for 10 min in 7 ml of toluene/ethyl acetate (v/v). After extraction, the aqueous phase was frozen with liquid nitrogen, and the organic layer was poured into a scintillation vial to which 0.4 ml/h of 2,5-diphenyloxazole was subsequently added. After 5 min of agitation, radioactivity was measured in a scintillation spectrometer (LS-1801, LS-1701; Beckman Coulter Inc., Fullerton, CA). Blank values were obtained by adding HCl before the substrate. MAO activities were corrected for the efficiencies of extraction of deaminated metabolites into the toluene/ethyl acetate phase (for [14C]PEA, 0.95; for [14C]5-HT, 0.77). IC50 values were calculated from inhibition curves, and IC50 is expressed with various concentrations of inhibitor in a total volume of 400 µl.

**Assay of Brain Catecholamines, 5-HT, and Their Related Metabolites**

Animals were killed by decapitation at specified times after oral administration of SL25.1131. Striata and frontal cortex were dissected out, frozen, weighed, and stored at −80°C until analysis. NE, 5-HT, DA, HVA, DOPAC, NMN, 3-MT, and 5-HIAA were quantified in the supernatant by high-pressure liquid chromatography with electrochemical detection and were assayed as described previously (Aubin et al., 1998).

**Interaction with Tyramine in Awake Rats**

Arterial pressure was directly measured in awake freely moving rats (n = 11–20) prepared with indwelling cannulas in the carotid artery as described previously (Caille et al., 1996). MAOIs were studied at equipotent pharmacological doses, i.e., at least three times the oral ED50 values determined in the rat 1.5-HTP potentiation-test (Table 3). Pretreatment time for MAOIs was chosen to produce a maximal peripheral inhibition of MAO. Tyramine responses were expressed as the maximal changes in ΔSBP maximal, in millimeters of mercury, the most sensitive parameter for the evaluation of a hypertensive effect, calculated versus the control value [just before the tyramine (12 mg/kg p.o.) administration]. Data were expressed as mean ± S.E.M.

**Unilateral Lesion of the Mesotelencephalic Dopaminergic System in Rats**

The mesotelencephalic system was lesioned unilaterally by two injections of 6-OHDA (6 µg in 1.5 µl; vehicle, 0.9% saline and 0.01% ascorbic acid) in the left median forebrain bundle (stereotaxic coordinates relative to bregma: AP, 0–1; ML, +1.6; V, −8 from dura; incisor bar, +5), as described previously (Barneveld et al., 2000). Rats were screened for the presence of a total dopaminergic lesion by examining their circling response to 50 µg/kg apomorphine hydrochloride (see rotation activity section). Rats that made fewer than 150 contralateral rotations over 45 min were excluded.

**Behavioral Testing**

**Y-Maze Activity.** Locomotor and rearing activities in mice were determined in a symmetrical Y-maze as described by Marks et al. (1985). The maze consisted of three arms 26 cm in length, 6.1 cm in width, and 15 cm in height. Each arm was subdivided into two equal
Fig. 2. Time course of the effects of systemic administration of SL25.1131 on the ex vivo activity of MAO-A (■) and MAO-B (●) in the rat frontal cortex, striatum, liver, and duodenum. Animals were sacrificed at various times after the administration of SL25.1131 (3.5 mg/kg p.o.), and MAO-A and MAO-B activities were measured in homogenates. The results are expressed as percentage of variation versus respective controls. Each point represents the mean value ± S.E.M.; n = 6 animals/group. * p < 0.05 compared with respective control. Control activities (nanomoles per minute per gram of tissue) were (MAO-A/MAO-B) frontal cortex, 214 ± 67/67; striatum, 200 ± 47/47; liver, 478 ± 10/10; and duodenum, 166 ± 8/8 compared with control rats.

TABLE 1
In vitro MAO-A and MAO-B inhibition by SL25.1131 and various MAO inhibitors in rat brain homogenates
The confidence intervals are shown in parentheses.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC₅₀</th>
<th>MAO-A</th>
<th>MAO-B</th>
<th>B/A</th>
</tr>
</thead>
<tbody>
<tr>
<td>SL25.1131</td>
<td>6.7 (6.0–7.9)</td>
<td>16.8 (14.9–18.9)</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>Befloxatone*</td>
<td>3.8 (3.6–4.1)</td>
<td>300 (275–361)</td>
<td>79</td>
<td></td>
</tr>
<tr>
<td>Cimoxatone*</td>
<td>2.7 (2.6–2.9)</td>
<td>90 (83–99)</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>Harmaline*</td>
<td>12 (10–14)</td>
<td>&gt;1000</td>
<td>&gt;83</td>
<td></td>
</tr>
<tr>
<td>Moclobemide*</td>
<td>23,000 (21,000–26,000)</td>
<td>&gt;10,000</td>
<td>&gt;0.5</td>
<td></td>
</tr>
<tr>
<td>BW1370U87*</td>
<td>50 (46–54)</td>
<td>&gt;1000</td>
<td>&gt;20</td>
<td></td>
</tr>
<tr>
<td>Toloxatone*</td>
<td>3260 (2860–3660)</td>
<td>39,000 (31,000–50,500)</td>
<td>&gt;12</td>
<td></td>
</tr>
<tr>
<td>L-Deprenyl*</td>
<td>970 (864–1000)</td>
<td>4.6 (3.9–5.5)</td>
<td>&lt;0.005</td>
<td></td>
</tr>
<tr>
<td>Lazabemide*</td>
<td>&gt;10,000</td>
<td>30 (25–39)</td>
<td>&lt;0.005</td>
<td></td>
</tr>
</tbody>
</table>

* Data from Curet et al. (1996, 1998).

TABLE 2
Ex vivo MAO-A and MAO-B inhibition by SL25.1131 in rat brain homogenates: comparison with other MAO inhibitors
The confidence intervals are shown in parentheses.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>ED₅₀</th>
<th>MAO-A</th>
<th>MAO-B</th>
<th>B/A</th>
</tr>
</thead>
<tbody>
<tr>
<td>SL25.1131</td>
<td>0.67 (0.52–0.82)</td>
<td>0.52 (0.40–0.64)</td>
<td>0.8</td>
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<tr>
<td>Befloxatone*</td>
<td>0.02 (0.01–0.04)</td>
<td>1.2 (0.9–1.8)</td>
<td>60</td>
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</tr>
<tr>
<td>Moclobemide*</td>
<td>1 (0.8–1.2)</td>
<td>&gt;120</td>
<td>&gt;120</td>
<td></td>
</tr>
<tr>
<td>Clorgyline*</td>
<td>3.4 (3.8–3.0)</td>
<td>&gt;20</td>
<td>&gt;6</td>
<td></td>
</tr>
<tr>
<td>Tranylcypromine*</td>
<td>0.5 (0.43–0.56)</td>
<td>0.24 (0.21–0.27)</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Phenelzine*</td>
<td>6.2 (5.5–7.4)</td>
<td>12 (8.4–15.5)</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Pargyline*</td>
<td>9.3 (8.2–10.5)</td>
<td>1.4 (1.3–1.7)</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>Nialamide*</td>
<td>13 (11–15)</td>
<td>57 (47–68)</td>
<td>4.4</td>
<td></td>
</tr>
<tr>
<td>L-Deprenyl*</td>
<td>&gt;30</td>
<td>1.5 (1.6–1.4)</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>Lazabemide*</td>
<td>&gt;1</td>
<td>0.065 (0.04–0.11)</td>
<td>&lt;0.065</td>
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</tr>
</tbody>
</table>

* Data from Curet et al. (1998).
sections. The mice were placed individually in the center of the maze, and movements from one section to another were counted over a 4-min period.

1-L-5-HTP-Induced Tremors. The potentiation of 1-L-5-HTP was assessed according to the method of Lessin (1959). Groups of mice received p.o. injections of the test compounds or the vehicle, followed 60 min later by 1-L-5-HTP (100 mg/kg i.p.). This dose did not induce tremors in control animals. Immediately after receiving 1-L-5-HTP, mice were placed individually in clear polystyrene cages (22 × 10 × 8 cm). Thirty minutes after 1-L-5-HTP, they were individually observed for 30 s for the presence of generalized tremor, which was scored as either present or absent. The activity of the test compounds was expressed as the dose that produced generalized tremors in 50% of the animals (ED50) as calculated by log-probit analysis of the linear part of the dose-response curve.

L-DOPA-Induced Activity. MPTP-treated mice were placed individually in clear polystyrene cages (22 × 10 × 8 cm) and were observed individually for 30 s for the presence or absence of locomotor activity, rearing or stereotypes. Mice were recorded as positive if at least one of these behaviors was observed.

L-DOPA-Induced Rotation. Rats were tested in an automated rotameter (MED Associates, St. Albans, VT) and the number of full 360° turns per 5-min interval was recorded automatically by microcomputer. Three weeks after surgery, animals were treated with L-DOPA and tested in rotameters, and the number of full turns per minute was recorded during a 480-min period. Rotations in the ipsilateral and contralateral directions were counted separately, and the analyses were based on the net scores (contralateral minus ipsilateral rotations); a positive score indicated that animals exhibited a net contralateral bias, whereas a negative score indicated a net ipsilateral bias.

Drug Treatments

SL25.1131, tolcapone, L-DOPA, and benserazide were suspended in 0.5% (w/v) methocel gel in sterile water with the addition of 0.05% Tween 80. Control groups received the vehicle used for SL25.1131 (0.5% methocel gel plus 0.05% Tween 80). PEA, 1-L-5-HTP, 6-OHDA, and MPTP were dissolved in physiological saline, and the doses refer to the salt. For all other drugs, the doses always refer to the free base and are expressed in milligrams per kilogram of body weight. Drugs were administered in a volume of 5 and 10 ml/kg in rats and mice, respectively. Tolcapone and SL25.1131 were administered orally; L-DOPA and benserazide were administered intraperitoneally. MPTP was administered in saline subcutaneously.

Statistical Analysis

IC50, ED50, and confidence intervals were calculated with a RS1 procedure (Utistat-dose effect). When appropriate, drug effects were assessed by comparing drug-treated animals with control animals using either a Student’s t test for single comparison or an analysis of variance (one or two factors) followed by the Dunnett’s (homogenous variances) or the Bonferroni-Holm (heterogeneous variances) tests (Utistat, RS1, Everstat) or Newman-Keuls or a nonparametric Kruskal-Wallis test. The L-DOPA-induced hyperactivity in MPTP-lesioned mice was subjected to a chi square analysis and Fisher’s exact test.

Results

Inhibition by SL25.1131 of MAO-A/B Activity in Rat Brain Homogenates in Vitro. In vitro, SL25.1131 induced a potent and nonselective inhibition of MAO-A and MAO-B activities in rat brain homogenates with IC50 values (confidence interval within parentheses) of 6.7 (6.0–7.9) nM and 16.8 (14.9–18.9) nM, respectively (Table 1).

For purposes of comparison, IC50 values of reversible, selective MAO-A inhibitors, including befloxatone, cimoxatone, harmaline, moclobemide, brofaromine, BW1370U87, and toloxatone, and selective MAO-B inhibitors, including lazabemide (reversible) and l-depenyl (irreversible) (Curet et al., 1996, 1998), are also presented in Table 1.

With low-affinity inhibitors, the amount of compound needed to inhibit enzymatic activity is relatively large compared with the amount of enzyme. Consequently, the amount of inhibitor bound to the enzyme represents a negligible fraction and the concentration of free inhibitor can be taken...
as equal to the total concentration of inhibitor. Under these conditions, the Michaelis equation can be used.

In contrast, with very efficacious inhibitors (K_i values of the order of 10^{-10} M), including SL25.1131, the concentration of inhibitor and enzyme may be of the same order. In this case, the binding of the inhibitor to the enzyme reduces the concentration of free inhibitor and the equation, which is used, requires modification of IC_{50} or K_i parameters, which cannot be calculated absolutely (Morrison, 1969). In addition, standard experimental conditions for determination of IC_{50} require optimization (preincubation period). For these reasons, Kiapp, a parameter that takes into account the MAO-A and MAO-B concentrations (see Materials and Methods) has been calculated.

In rat brain, Kiapp of SL25.1131 was 3.31 (0.07–0.07) nM for MAO-A and 4.18 (0.06–0.06) nM for MAO-B. Control activities for MAO-A and MAO-B were 170 and 58 pmol/min/mg tissue, respectively.

Effect of SL25.1131 on MAO-A and MAO-B Activities in Rat Brain ex Vivo. SL25.1131, 1 h after oral administration, induced a dose-dependent inhibition of MAO-A and MAO-B activities in the rat brain with ED_{50} values of 0.67 (0.52–0.82) and 0.52 (0.40–0.64) mg/kg, respectively (Table 2). A dose of 10 mg/kg p.o. SL25.1131 was necessary for the full inhibition of MAO-A and -B activities in rat and mouse brains (data not shown).

For purposes of comparison, ED_{50} values of selective MAO-A inhibitors, including befloxatone (reversible), moclobemide (reversible), and clorgyline (irreversible), selective MAO-B inhibitors, including lazabemide (reversible) and t-deprenyl (irreversible), and irreversible mixed MAO inhibitors, including tranylcypromine, phenelzine, pargyline, and nialamide (Curet et al., 1998), are also presented in Table 2.

Time course of the effect of systemic administration of SL25.1131 (3.5 mg/kg p.o.), administered 1, 2, 4, 8, 16, and 24 h before decapitation, on MAO-A and MAO-B activities in rat frontal cortex, striatum, liver, and duodenum is shown in Fig. 2. SL25.1131 (3.5 mg/kg p.o.) induced a maximal inhibition of MAO-A (80 to 85%) and MAO-B (approximately 95%) at 1 h after administration. The inhibition of both isoforms of MAO was fully reversible, and the recovery of MAO-A and MAO-B activities was complete at 16 h.

### Table 3

<table>
<thead>
<tr>
<th>Compounds</th>
<th>ED_{50} (mg/kg; p.o.)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rat</strong></td>
<td><strong>Mice</strong></td>
</tr>
<tr>
<td>t-5-HTP Tremor</td>
<td>t-5-HTP Tremor</td>
</tr>
<tr>
<td>SL25.1131</td>
<td>0.61 (0.55–0.69)</td>
</tr>
<tr>
<td>Befloxatone*</td>
<td>0.15 (0.11–0.21)</td>
</tr>
<tr>
<td>Moclobemide*</td>
<td>1.6 (1.1–2.1)</td>
</tr>
<tr>
<td>Brofaromine*</td>
<td>6.9 (4.2–9.5)</td>
</tr>
<tr>
<td>Tranylcypromine*</td>
<td>16 (14–18)</td>
</tr>
<tr>
<td>Nialamide*</td>
<td>20 (15–29)</td>
</tr>
<tr>
<td>Phenelzine*</td>
<td>&gt;128</td>
</tr>
<tr>
<td>t-Deprenyl*</td>
<td>&gt;128</td>
</tr>
</tbody>
</table>

* Data from Caille et al. (1996).
Effects of SL25.1131 on the Levels of Monoamines and Their Metabolites in the Rat Frontal Cortex and Striatum. As shown in Fig. 3, SL25.1131 (3.5 mg/kg p.o.), in the rat frontal cortex, induced a maximal increase of NE (32%), 5-HT (100%), and NMN (250%) levels and a maximal decrease of DOPAC (−70%) and HVA (−55%) levels 1 h after administration. In this region, levels of 5-HIAA were not significantly modified.

A similar pattern was observed in the striatum where a substantial increase of DA (25%), 5-HT (40%), and 3-MT (150%) levels and a significant decrease of DOPAC (−80%), HVA (−75%), and 5-HIAA (−20%) levels were observed 1 h after dosing. In both regions, the levels of monoamines and their metabolites returned to control values between 8 and 24 h.

Potentiation of L-5-HTP and PEA-Induced Behaviors by SL25.1131. Systemic administration of L-5-HTP induces tremors in mice due to its 5-HT-releasing effect. Likewise, systemic administration of PEA induces hyperactivity and tremors in mice due to its 5-HT-releasing effect. The potentiating effects of oral administration of SL25.1131 on L-5-HTP-induced tremors in rats and in mice and PEA-induced stereotypies in mice are shown in Table 3.

The ED50 (confidence interval within parentheses) for potentiation of L-5-HTP-induced tremors by SL25.1131 is 0.61 (0.55–0.69) mg/kg p.o. and 0.60 (0.55 to 0.67) mg/kg p.o., in rats and in mice, respectively. In mice, the ED50 (confidence interval within brackets) for potentiation of PEA-induced stereotypies by SL25.1131 is 2.8 (2.7–3.0) mg/kg p.o. For purposes of comparison, ED50 values of a variety of reference compounds (Curet et al., 1998), are also presented in Table 3, demonstrating the specificity of the L-5-HTP-induced tremors and PEA-induced stereotypies for MAO-A, and MAO-B activities, respectively.

Interaction of SL25.1131 with Tyramine in the Awake Rat. As shown in Fig. 4, after pretreatment (60 min) with SL25.1131 (3.3 mg/kg p.o.), tyramine (12 mg/kg p.o.) did not alter SBP (ASBP maximal = 22 ± 6 compared with 20 ± 8 mm Hg in the vehicle-treated group).

For purposes of comparison, the effects of other reversible MAO-A inhibitors, including befloxatone, moclobemide, and brofaromine, and irreversible mixed MAO inhibitors, including nialamide and phenelzine (Caille et al., 1996), are also presented in Fig. 4. The tested doses of MAOIs were determined in accordance with their relative in vivo activity in rats, i.e., at least three times the oral ED50 values determined in the rat L-5-HTP potentiation test (Table 3). Pretreatment time for MAOIs was chosen to produce a maximal peripheral inhibition of MAO: 60 min for SL25.1131 (3.3 mg/kg p.o.), 45 min for befloxatone (0.5 mg/kg p.o.), 30 min for moclobemide (5 mg/kg p.o.), 60 min for brofaromine (21 mg/kg p.o.), nialamide (60 mg/kg p.o.), and phenelzine (48 mg/kg p.o.).

As shown in Fig. 4, when SL25.1131 or other reversible MAOIs were given before the tyramine challenge (12 mg/kg p.o.), no modification of the effect of tyramine was observed, whereas the irreversible MAOIs phenelzine (48 mg/kg p.o.) and nialamide (60 mg/kg p.o.) produced a marked potentiation of the pressor effect with tyramine under the same conditions.

Effects of SL25.1131 on Striatal Levels of DA, DOPAC, 3-MT, and 5-HT in MPTP-Lesioned Mice. As shown in Fig. 5, MPTP (40 mg/kg s.c.) decreased DA, DOPAC, and 3-MT levels by −90, −83, and −78%, respectively, and was without effect on 5-HT levels.

In MPTP-lesioned mice, SL25.1131 (10 mg/kg p.o.) increased 3-MT and 5-HT levels by 400 and 55%, respectively, and decreased levels of DOPAC by −86% without significant modification of DA levels.

Effects of SL25.1131 Alone or in Combination with L-DOPA on Striatal Levels of DA, DOPAC, 3-MT, and 5-HT in MPTP-Lesioned Mice. In MPTP-lesioned mice, different doses of L-DOPA (25, 50, and 100 mg/kg i.p. + benserazide 25 mg/kg i.p.) increased striatal levels of DA (228, 243, and 460%), DOPAC (594, 852, and 2165%), and 3-MT (119, 98, and 182%) but decreased levels of 5-HT (−22, −26, and −51%) (Fig. 6). Compared with MPTP groups, administration of different doses of L-DOPA (i.p.) in combination with a dose of SL25.1131 (10 mg/kg p.o.) increased the levels of DA (349, 873, and 591%), 3-MT (1019, 1205, and 2733%), and 5-HT (51, 41, and 32%) (Fig. 6). These variations were dose-dependent, except for DA. Furthermore, SL25.1131 had no effect on striatal L-DOPA levels in MPTP-lesioned mice (data not shown).

Effects of SL25.1131 on MPTP-Induced Hypoactivity in Mice. Forty-eight hours after a single MPTP administration, locomotion was reduced in mice by 30% [Fig. 7; F(3,36) = 6.59; p = 0.001]. At the same time, striatal DA levels were decreased by approximately 80%. It is probable that this hypoactivity is closely related to the loss of striatal DA terminals, because the coadministration of the reuptake blocker GBR12935 with MPTP counteracts both the penetration of the toxin 1-methyl-4-phenylpyridinium into dopaminergic neurons and the hypoactivity (data not shown).

SL25.1131, administered 1 h before the behavioral evaluation (a period at which the lesion was completed), was able to reestablish, in lesioned-mice, a locomotion similar to that exhibited by vehicle-treated control mice. In addition, SL25.1131 treatment did not change this behavior in nonlesioned mice.

Effects of SL25.1131 and Tolcapone on L-DOPA-Induced Activity in MPTP-Lesioned Mice. L-DOPA treatment increased in a dose-dependent manner the number of animals that demonstrated general activity (without treatment, mice remained motionless because of the habituation to the cage). The active doses were 25 and 50 mg/kg, and the subactive dose was 12.5 mg/kg (Fig. 8A). Pretreatment with SL25.1131 (10 mg/kg p.o.) potentiated the effect of L-DOPA (12.5 mg/kg) (from 20 to 70% responders), whereas tolcapone (30 mg/kg p.o.) failed to have any effect (Fig. 8A). Pretreatment with SL25.1131 maintained the therapeutic effect with half the dose of L-DOPA (Fig. 8A). Moreover, as illustrated in Fig. 8B, pretreatment with SL25.1131 (10 mg/kg p.o.) or tolcapone (30 mg/kg p.o.) increased the duration of L-DOPA effect at doses of 12 and 25 mg/kg, respectively.

Effects of SL25.1131 and Tolcapone on L-DOPA-Induced Rotations in Rats with a Complete 6-OHDA Lesion. In 6-OHDA-lesioned rats, the combined administration of L-DOPA (5–20 mg/kg i.p.) and benserazide (15 mg/kg i.p.) induced contralateral circling behavior (Fig. 9A), and the L-DOPA treatment increased in a dose-dependent manner the duration
of turning behavior \( F(94,3572) = 8.23; p < 0.001 \) and the total number of turns \( F(2,76) = 9.9; p < 0.001 \). Administration of either SL25.1131 (10 mg/kg p.o.) or tolcapone (30 mg/kg p.o.) increased the duration \( F(270,3060) = 4.0; p < 0.001 \) and the total number of turns \( F(3,34) = 13.36; p < 0.001 \) induced by a dose of 5 mg/kg L-DOPA (Fig. 9B). Combined inhibition of both the MAO and COMT inhibitors dramatically potentiated L-DOPA-induced turning in comparison with MAO or COMT inhibition alone.

Discussion

In vitro, with IC\(_{50}\) values of 6.7 and 16.8 nM and Kiapp values of 3.3 and 4.2 nM for rat brain MAO-A and MAO-B, respectively, SL25.1131 is a potent, mixed, and competitive MAO inhibitor. In contrast to its potent inhibitory effect on MAO-A/B, SL25.1131 is devoid of any activity (Ki > 1 \( \mu \)M) at 100 different receptor binding sites studied (CEREP binding profiles; data not shown). Ex vivo, SL25.1131 induced a dose-dependent and reversible inhibition of MAO-A and MAO-B activities in rat brain with ED\(_{50}\) values of 0.67 and 0.52 mg/kg p.o., respectively. Its inhibitory activity is similar to that of tranylcypromine and SL25.1131 is 10- to 20-fold more potent than phenelzine.

The pattern of the effects of SL25.1131 on monoamine levels and their metabolites is that expected for a reversible MAO-A/B inhibitor, with an increase of tissue levels of monoamines associated with a decrease of their deaminated metabolites and an increase of their methylated metabolites. Monoamine levels and metabolites returned to basal values 8 to 16 h after dosing. This time course paralleled those of the inhibition of MAO-A and MAO-B activities, further demonstrating that SL25.1131 is a reversible MAO inhibitor in vivo.

Because selective MAO-B inhibitors do not affect monoamine levels and their metabolites in the rat brain, the variations observed after systemic administration of SL25.1131 are likely mainly due to MAO-A inhibition (Kan et al., 1987; Da Prada et al., 1989; Curet et al., 1996). However, because previous reports demonstrated that monoamines can also be deaminated by MAO-B when MAO-A is inhibited (Butcher et al., 1990), variations in monoamine levels after administration of SL25.1131 could be due to inhibition of both isoforms of MAO.

The behavioral syndromes induced by 5-5-HTP or PEA are potentiated by MAO-A and MAO-B inhibition, respectively (Jacobs, 1976; Ortmann et al., 1980, 1984), and these functional tests can thus be used to evaluate the selectivity of inhibition of either enzyme isoform in vivo. The ED\(_{50}\) values for inhibition of MAO-A activity and of 5-5-HTP-induced tremors in mice (0.67 and 0.60 mg/kg p.o., respectively) (Tables 2 and 3) are of the same order of magnitude. In addition, SL25.1131 potentiated PEA-induced stereotypies in mice with an ED\(_{50}\) of 2.8 mg/kg p.o. (Table 3), while decreasing
MAO-B activity with an estimated ED$_{50}$ of 0.52 mg/kg p.o. (Table 2). These results confirm the in vivo activity of SL25.1131 as an inhibitor of MAO-A and MAO-B.

In this study, we investigated the interaction of SL25.1131 with oral tyramine effects in freely moving awake rats according to Caille et al. (1996). When SL25.1131 (at a dose that fully inhibited MAO-A and MAO-B activities) was given 1 h before the tyramine challenge (12 mg/kg p.o.), no change in the pressor response induced by tyramine was observed. A similar lack of tyramine potentiation was observed with reversible vehicles. The MPTP group received MPTP and the appropriate vehicle. The results are expressed as percentage of change versus controls (mean ± S.E.M.; n = 6). Control levels (nanograms per gram of frozen tissue) were DA, 14,796 ± 419; DOPAC, 3327 ± 260; 3-MT, 1105 ± 69; and 5-HT, 725 ± 59. *p < 0.05 versus respective control (analysis of variance two-way).

Fig. 6. Ex vivo effect of different doses of L-DOPA (25, 50, and 100 mg/kg i.p.)/benserazide (25 mg/kg i.p.) alone or in combination with SL25.1131 (10 mg/kg p.o.) on tissue levels of DA, DOPAC, 3-MT, and 5-HT in the striatum of C57BL/6 mice lesioned by a single injection of MPTP (40 mg/kg s.c.) 8 days before. The animals were sacrificed 1 h after the administration of SL25.1131 and 30 min after the administration of L-DOPA/benserazide. The control group was injected with appropriate vehicles. The MPTP group received MPTP and the appropriate vehicle. The results are expressed as percentage of change versus controls (mean ± S.E.M.; n = 6). Control levels (nanograms per gram of frozen tissue) were DA, 14,796 ± 419; DOPAC, 3327 ± 260; 3-MT, 1105 ± 69; and 5-HT, 725 ± 59. *p < 0.05 versus respective control (analysis of variance two-way).
COMT dramatically potentiated the duration of L-DOPA-induced turning in comparison with MAO or COMT inhibitions only. Our results are in accordance with those Heeringa et al. (1997) who demonstrated that inhibition of both MAO-A (Ro41-1049) and MAO-B (Ro19-6327) potentiated the duration of L-DOPA rotations. Together, these results demonstrate that inhibition of MAO-A and MAO-B by SL25.1131 increased DA transmission in MPTP or 6-OHDA-lesioned animals alone and in combination with L-DOPA.

L-DOPA remains the most effective treatment for PD, even if it is associated with a number of problems (i.e., motor fluctuations and dyskinesias) after only 5 to 7 years of use. Many clinicians favor delaying the introduction to L-DOPA until symptoms are severe enough to warrant its use (Fahn, 1996), particularly in younger patients, and DOPA-sparing strategies are often used, mainly using other antiparkinsonian drugs first and keeping the dosage of L-DOPA as low as possible when it is used. MAO mixed inhibitors, which reduce the metabolism of DA, could alleviate the motor dysfunctions in PD patients when administered alone (Fahn and Chouinard, 1998). As we demonstrated here, the reversible, mixed MAO inhibitor SL25.1131 increased striatal dopaminergic transmission in partial MPTP-lesioned mice, the levels of the extracellular DA metabolite 3-MT reaching normal values, and reestablished in the lesioned mice a locomotion similar to that exhibited by control mice. The treatment of patients with early PD by selegiline could delay the introduction of L-DOPA by about 9 months (Parkinson Study Group, 1996a). To provide possible benefit by inhibition of both MAO types A and B, Fahn and Chouinard (1998) offered patients with mild PD not requiring symptomatic therapy the choice to be treated with tranylcypromine. The authors found a lower rate of reaching endpoint (the time when symptomatic treatment is needed) in tranylcypromine-treated patients compared with selegiline-treated patients, suggesting that inhibiting both MAO-A and -B may have merit as a symptomatic treatment in early PD.

As PD progresses, the use of L-DOPA becomes increasingly necessary. Whether the motor complications seen with chronic L-DOPA therapy in patients with PD are actually caused by long-term L-DOPA therapy or simply reflect the progression of the disease is unknown. Nevertheless, the frequency of motor fluctuations and dyskinesias could result in part from the pulsatile stimulation of receptors caused by intermittent dosages of standard L-DOPA preparations (Chase et al., 1993). However, more frequent smaller doses may cause more off time and dose failures (Metman et al., 1997), and alternative formulations and routes of delivery developed to lengthen the relatively short half-life of conventional preparations of L-DOPA are difficult to use because of reduced bioavailability (Koller and Pahwa, 1994) and their pharmacokinetic profiles (Hughes, 1997). Thus, drugs designed to extend the duration of the response to L-DOPA should regulate these fluctuations.

**Fig. 7.** Effects of SL25.1131 on MPTP-induced hypoactivity in mice. Mice were injected with MPTP (40 mg/kg s.c.) or saline, and their motor behavior was studied 2 days later, and 60 min after SL25.1131 (10 mg/kg p.o.) treatment (8 animals/group) or vehicle. Mice were subdivided into four groups according to the treatments they received: saline + vehicle (n = 16); saline + SL25.1131 (n = 8); MPTP + vehicle (n = 8); and group MPTP + SL25.1131 (n = 8). Results are expressed as mean number of crossings (mean ± S.E.M.); **p < 0.01.

**Fig. 8.** Effects of SL25.1131 and tolcapone on L-DOPA-induced hyperactivity in MPTP-lesioned mice. Mice were injected with MPTP (40 mg/kg s.c.), and their activity was studied 7 days later. A, L-DOPA dose-response curve. B, L-DOPA dose-duration relationship. Mice were subdivided into groups according to the treatment they received: vehicle + L-DOPA (6, 12, 25, and 50 mg/kg i.p.; n = 12); vehicle + SL25.1131 with L-DOPA (3, 6, 12, and 25 mg/kg i.p.; n = 12); and tolcapone + vehicle + L-DOPA (6, 12, 25, and 50 mg/kg i.p.; n = 12). Benserazide (25 mg/kg i.p.) was added to L-DOPA. SL25.1131 and tolcapone were administered 30 and 90 min before L-DOPA, respectively. The behaviors were studied 30 min, 1 h, 2 h, 3 h, 4 h, 5 h, and 6 h after L-DOPA treatment. Results are expressed as mean time (mean ± S.E.M.); *, p < 0.05 and **, p < 0.01 versus the "vehicle + vehicle" group.
tolcapone and entacapone have been used, and they can prolong the action of an individual dose of L-DOPA and significantly reduce “off” time (Kurth et al., 1997). However, they also increase L-DOPA side effects such as dyskinesias or hallucinations, perhaps because they enhance the level of cerebral L-DOPA. By blocking the degradation of DA, the reversible mixed MAO inhibitor SL25.1131 potentiated the increase in striatal dopaminergic transmission induced by L-DOPA in MPTP-lesioned mice without changing the level of cerebral L-DOPA (see Results). In addition, it potentiated L-DOPA-response in animal models of PD, reducing by half the dose of L-DOPA necessary for their therapeutic effect and increasing the duration of this effect. Because our compound is devoid of activity on L-DOPA metabolism, we are tempted to speculate that, if coadministered with a low dose of L-DOPA in early PD, it would stabilize the L-DOPA fluctuations at the start of the treatment, and it would reduce the frequency of the future L-DOPA side effects (i.e., dyskinesias and motor fluctuations).

In conclusion, the neurochemical profile of SL25.1131 demonstrates that this compound is a mixed, potent, and reversible MAO-A and MAO-B inhibitor in vitro, ex vivo, and in vivo, which exhibits in animals a better safety index than irreversible, mixed MAOs. SL25.1131 was able to reestablish normal striatal dopaminergic tone and locomotor activity in MPTP-lesioned mice. In addition, when coadministered with L-DOPA, SL25.1131 optimized the effect of L-DOPA by increasing the available DA in the striatum and the duration of its effect. A study in nonhuman primate would be envisaged to reinforce our data in rodents. Thus, SL25.1131 has therapeutic potential as a symptomatic treatment during the early phase of the PD and as an adjunct to L-DOPA therapy during the early and late phases of the disease.

Acknowledgments
We thank Dr. D. Sanger for critically reading the manuscript.

References


References

SL25.1131, a New, Reversible, and Mixed MAO-A/B Inhibitor 1181

Fig. 9. Effect of SL25.1131 (10 mg/kg p.o.) and tolcapone (30 mg/kg p.o.) alone or in combination on L-DOPA-induced rotations in complete 6-OHDA-lesioned rats. A, dose-dependent effect of L-DOPA. One week after the apomorphine test, different doses of L-DOPA + benzerazide (15 mg/kg) were administered i.p., and rotation rate was measured over a 240-min period. Three groups of rats (n = 9 each) were treated with the three doses of L-DOPA (5, 10, and 20 mg/kg i.p.), each rat having received the three doses after 3 weeks of testing. B, effects of SL25.1131 and tolcapone coadministered with a low dose (5 mg/kg i.p.) of L-DOPA. Animals were subdivided into four groups according to the treatment they received before the administration of a low dose of L-DOPA (5 mg/kg + benzerazide 15 mg/kg i.p.): group V + V, vehicle + vehicle (n = 8); group V + SL, vehicle + SL25.1131 (n = 11); group T + V, tolcapone + vehicle (n = 8); and group T + SL, tolcapone + SL25.1131 (n = 11); SL25.1131 and tolcapone were administered 60 and 90 min before L-DOPA, respectively. Rotation rate was measured over a 450-min period. Results are expressed as mean rotation rate (mean ± S.E.M.).


