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Restorative effects of glutamate antagonists in experimental parkinsonism

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Summary. Several compounds with antagonistic actions on N-methyl-D-aspartate (NMDA) receptors were tested for an antiakinesic action in hypoactive MPTP-treated C57 BL/6 mice rendered tolerant to the motor activity enhancing effects of the 20 mg/kg, s.c., dose of L-Dopa; each compound was administered 60 min before the administration of the dopamine precursor. The classes of compounds studied included the noncompetitive NMDA antagonists, memantine, amantadine and MK-801, the competitive NMDA antagonist, CGP 40116, the anticonvulsive and putative anticonvulsive agents, lamotrigine and FCE 26743, with a partial glutamatergic antagonistic action. All six compounds elevated locomotor, rearing and total activity counts of L-Dopa-tolerant mice in co-administration with L-Dopa in dose-specific or dose-dependent manners but only memantine and MK-801 affected motor activity in the control mice, that also received chronic L-Dopa treatment. Thus, the restorative actions of those compounds in suprathreshold L-Dopa-tolerant MPTP-treated mice subjected to "wearing-off" of L-Dopa efficacy were assessed in a series of experiments. Within each class of potentially therapeutic agents a differential restorative efficacy of the motor activity-stimulating effects of hypoactive MPTP mice was obtained, confirming the putative antiparkinsonian applications of compounds with glutamate antagonistic actions.

Keywords: MPTP – Hypokinesia – Locomotion – Rearing – Memantine – Amantadine – MK-801 – CGP 40116 – Lamotrigine – FCE 26743 – Chronic administration – L-Dopa −20 mg/mg – Co-administration – Hyperactivity – Motor fluctuations – "Wearing-off" – Drug-tolerance – Synergism – Restoration – C57 BL/6 mice

Introduction

Severe loss of dopamine (DA) neurons in the substantia nigra (>80%) is a major feature of idiopathic Parkinson's disease (PD), characterised by bradykinesia, rigidity and tremors, accompanied by the depletion of DA concentrations in the striatum. Traditionally, dopaminergic therapies aimed at symptomatic control have been applied to initiate and execute locomotion (or a similarly assigned variable) both in parkinsonian patients and in the available animal models (cf. Levy et al., 1995; Gerlach and Riederer, 1996: Animal models of Parkinson's disease). Findings of L-Dopa utility for the treatment of PD patients (Barbeau, 1969), were contrasted with a diminished duration/magnitude of the antiparkinsonian motor behaviour effects following L-Dopa, i.e. "wearing-off" fluctuations or "drug-tolerance" observed both in the animal model (Fredriksson et al., 1999b) and in the clinical setting (Nutt et al., 1994). In common marmosets with near-complete unilateral 6-hydroxydopamine-induced denervation of DA inputs and DA depletion caused a profound supersensitization to L-Dopa treatment in the cerebral cortex and striatal complex as indexed by c-fos messenger RNA as biochemical marker of post-synaptic neuronal activation (Svenningsson et al., 2000). It was suggested that the effects of L-Dopa probably depend both on a direct activation of the supersensitized DA receptors by DA produced in the few remaining, hyperactive DA terminals and on indirect actions of L-Dopa involving activation of cerebral cortex – basal ganglia circuits (ibid).

One of the major pitfalls connected with chronic treatment with L-Dopa is the fluctuations in its antiparkinsonian efficacy. "On-off" fluctuations of responses are attributed to a combination of pharmacokinetic, pharmacodynamic and receptor-adaptive alterations after long-lasting administration (Bravi et al., 1994). It has been suggested recently by Chase and colleagues that response fluctuations produced by chronic L-Dopa treatment involve increased phospho-

rylation of NMDA receptors in the striatum leading to increased ionic conductance (Bravi et al., 1994; Oh et al., 1998). If this were the case, a beneficial effect – i.e. attenuation of motor fluctuations following chronic L-Dopa treatment – may be expected from the concomitant application NMDA receptor antagonists. In fact, those studies were able to demonstrate that an NMDA antagonist did attenuate the shortening of an L-Dopa "therapeutic" effect, as measured by contralateral rotations, observed in rats with unilateral 6-hydroxydopamine lesions of the nigro-striatal system following repetitive administration, over 22 days of a threshold dose of L-Dopa plus carbidopa (Engber et al., 1994). Recently, similar effects were observed in the same model with 1-aminoadamantane (amantadine) which is currently used for the treatment of Parkinson's disease (PD) and has NMDA receptor antagonistic properties (Karcz-Kubicha et al., 1998). It is not clear at present whether the effects observed are specific for the animal model used (contralateral rotations) or could be extended to other models based on DA deficit such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treated mice.

A vast amount of experimental data now supports the contention of an interactive, mobilizing, often synergistic, effect of various N-methyl-D-aspartate (NMDA) antagonists, both competitive and noncompetitive, upon the activity-stimulating actions of L-Dopa, dopamine (DA) agonists and compounds that elevate DA neurotransmission in animals rendered hypokinesic or akinesic through an interruption of DA transmission (e.g. Danysz et al., 1994; Gossel et al., 1995; Morelli et al., 1992; Schmidt and Kretschmer, 1997). Thus, Svensson et al. (1991) demonstrated a synergistic interaction of preferential DA autoreceptor antagonists with (+)-5-methyl-10,11-dihydro-5H-dibenzocyclohepten-5,10-imine meleate ([+]MK-801) in the stimulation of locomotion. Also, either SDZ EAA494 or DL-(E)-2-amino-4-methyl-5-phosphono-3-pentanoic acid (CGP-37849), competitive NMDA antagonists, in combination with the mixed DA D1/D2 agonists, CI 201–678 or SDZ 205–152 reversed akinesia in monoamine-depleted OF1 mice in a dose-dependent fashion (Hughes et al., 1971), as did the combination of L-Dopa with either 3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP) or MK-801 (Klockgether and Turski, 1990). Skuza et al. (1994) reported that the uncompetitive NMDA antagonists, memantine (2.5 or 5.0 mg/kg) or amantadine (10 or 20 mg/kg) enhance locomotion produced by L-Dopa (50 or 100 mg/kg, plus benserazide, 100 mg/kg) but not bromocriptine (5 or 10 mg/kg). Such positive, often synergistic antiparkinsonian-like activity obtained by combination of L-Dopa and NMDA receptor antagonists has possible clinical importance, and is not only a therapeutic advantage.

"Wearing-off" variants of motor fluctuations have been suggested to reflect decline in vesicular DA storage following progressive degeneration of striatal DA terminals (Chase et al., 1993). Basal ganglia are invested by glutamatergic pathways with glutamate neurons projecting from the cerebral cortex to striatum and subthalamic nucleus, from the subthalamic nucleus to the internal segment of the globus pallidus (Albin et al., 1992). The regional site of glutamic agents upon dopaminergic function may be the neostriatum (St. Pierre and Bedard, 1995). Compounds that selectively affect glutamatergic mechanisms may strongly influence motor function (Albin et al., 1992; Starr, 1995). It has been suggested too that elevated glutamate system activity may contribute to changes modulating motor response aberrations (Engber et al., 1994). Thus, Marin et al. (1996) studied the effects of chronic administration (four weeks) of L-Dopa, by itself, or in co-administration with MK-801 upon the appearance of motor response aberrations in rats with induced parkinsonism after unilateral microinjection of 6-hydroxydopamine to the left hemisphere medial forebrain bundle. Four weeks after twice daily injections of L-Dopa (methyl ester, 25 mg/kg, together with benserazide, 6.25 mg/kg), the duration of the contralateral turning response to L-Dopa (generally observed after unilateral 6-OHDA) had declined by 37% and number of L-Dopa injections without effect had more than doubled. Co-administration of the glutamate antagonist blocked completely the response duration decline and the frequency of ineffectual L-Dopa administrations. In the present study, chronic administrations of L-Dopa (20 mg/kg), exceeding four weeks, to hypoactive MPTP mice were followed by motor activity tests of L-Dopa efficacy upon motor behaviour restoration alone or in co-administration with different NMDA antagonists.

MPTP induces parkinsonism in human and non-human primates (Langston, 1985), inducing the loss of substantia nigra cells in the pars compacta of adult animals. It was previously shown that injections of MPTP (2×40 mg/kg) in C57 BL/6 mice induced L-Dopa reversible hypoactivity (Fredriksson et al., 1990; Sundström et al., 1990), a less rigorous dose treatment,

e.g. 2 × 20, 25 or 30 mg/kg of MPTP has been found not to reduce motility in the C57 black mice although DA concentrations may indicate upto 50–80% reduction (Heikkila et al., 1989; Sonsalla and Heikkila, 1986; Weimuller et al., 1989). The parameters of MPTP treatment in this mouse strain are long-lasting (up to and above 52 weeks after treatment) with a good correlation between the functional defect, hypokinesia, the neurochemical concomitant, a severe depletion of DA, and a dose- and time-dependent recovery of several parameters of motor behaviour after treatment with L-Dopa (cf. Fredriksson and Archer, 1994; Fredriksson et al., 1990). Earlier studies demonstrated that the acute co-administration of a subthreshold dose of L-Dopa (5 mg/kg) with low doses of either the noncompetitive NMDA antagonist, MK-801, or the competitive antagonist, CGP 40116 produced a synergistic elevation of motor activity in MPTP-treated mice (Archer et al., 1996; Fredriksson et al., 1994a,b). Interestingly, also in this model an phenomenon analogous to "wearing off" following chronic L-Dopa treatment has been observed recently (Archer and Fredriksson, 1999; Fredriksson et al., 1999).

Hence, the purpose of this investigation was to assess the *restorative* effects of different doses of the three noncompetitive NMDA receptor antagonists, memantine, amantadine and MK-801, the competitive antagonist, CGP 40116, and the anticonvulsive agents with an antiglutamatergic action, to hypokinetic MPTP-treated mice. In order to do so, these compounds were co-administered with a suprathreshold dose of L-Dopa (20 mg/kg) to which tolerance had been developed over five weeks of chronic treatment with regular monitoring of motor activity parameters (cf. Fredriksson et al., 1999). MK-801 is considered one of the the most selective compounds of this type (Wong et al., 1986). CGP 40116 is a competitive antagonist at NMDA receptors that acts synergistically with subthreshold doses of L-Dopa in DA-depleted mice (Fredriksson et al., 1994a,b), similar to other compounds of this class (Maj[J] et al., 1993). Memantine shows good selectivity and an uncompetitive open-channel blockade (Chen and Lipton, 1997), whereas amantadine is poorly selective (Kornhuber et al., 1994; Parsons et al., 1993, 1996). Lamotrigine, chemically related to pyrimidine, is a potently active antiepileptic agent, with a long duration action and a favourable pharmacological profile (Lamb et al., 1985; Miller et al., 1986; Wheatley and Miller, 1989). The preclinical compound, FCE 26743, has an anticonvulsive action at relatively low doses (Dostert et al., 1991; Maj[R] et al., 1993) as well as being a potent, selective and irreversible MAOB inhibitor (Strolin Benedetti et al., 1994).

Methods and materials

Animals

In all the experiments described, three-to-six month old male C57 BL/6 mice (ALAB, Sollentuna, Sweden), weighing 22–25 g were used. Following arrival at the laboratory, the mice were allowed to acclimatize for two weeks in a room with controlled temperature (21 ± 1°C), and a constant light-dark schedule (12 hr on/12 hr off, lights on between 06.00 and 18.00 hrs). Free access to food and water was maintained throughout. They were housed in groups of 10 animals and tested only during the hours of light (08.00–15.00 hrs). All testing was performed in a normally lighted room. This test room, in which all 12 ADEA activity test chambers, each identical to the home cage, were placed, was well-secluded and used only for this purpose. Each test chamber (i.e. activity test cage) was placed in a sound-proofed wooden box with 12 cm thick walls and front panels and had a dimmed lighting. Experiments were carried out in accordance with the European Communities Council Directive of 24th November 1986 (86/609/EEC) after approval from the local ethical committee (Uppsala University and Agricultural Research Council), and by the Swedish Committee for Ethical Experiments on Laboratory Animals (license S93/92 and S77/94, Stockholm, Sweden).

Behavioural measurements and apparatus

An automated device, consisting of macrolon rodent test cages (40 × 25 × 15 cm) each placed within two series of infra-red beams (at two different heights, one low and one high, 2 and 8 cm, respectively, above the surface of the sawdust, 1 cm deep), was used to measure spontaneous and/or drug-induced motor activity of MPTP and control mice (RAT-O-MATIC, ADEA Elektronic AB, Uppsala, Sweden). The distance between the infra-red beams was as follows: the low level beams were 73 mm apart lengthwise and 58 mm apart breadthwise in relation to the test chamber; the high level beams, placed only along each longside of the test chamber were 28 mm apart. According to the procedures described previously (Archer et al., 1996), the following parameters were measured: LOCOMOTION was measured by the low grid of infra-red beams. Counts were registered only when the mouse in the horizontal plane, ambulating around the test-cage. REARING was registered throughout the time when at least one high level beam was interrupted, i.e. the number of counts registered was proportional to the amount of time spent rearing. TOTAL ACTIVITY was measured by a sensor (a pick-up similar to a gramophone needle, mounted on a lever with a counterweight) with which the test cage was constantly in contact. The sensor registered all types of vibration received from the test cage, such as those produced both by locomotion and rearing as well as shaking, tremors, scratching and grooming. All three behavioural parameters were measured over consecutive 30-min. periods.

Treatment and chemicals

MPTP (Research Biochemical Inc., Natick, MA. USA, 2 × 40 mg/kg, s.c., 24 hr interval, administered four to six weeks before behavioural testing, nine weeks in the case of the chronic administration

of L-Dopa experiments), L-Dopa (AB Hässle, Mölndal, Sweden), CGP 40116 (Ciba-Geigy AG, Switzerland), 1-aminoadamantane hydrochloride (amantadine, Aldrich, USA), 1-amino-3,5-dimethyl-adamantane hydrochloride (memantine, Merz + Co, Frankfurt/Mam, Germany) and (+)-5-methyl-10,11-dihydro-5H-dibenzo-cyclohepten-5,10-imine maleate (MK-801, Research Biochemicals, USA) were all dissolved in saline. Lamotrigine isothionate (3,5-diamino 6-[2,3-dichlorophenyl]-1,2,4-triazine), Batch No. AR 11227/17 and FCE 26743 [(S)-2–4-(3-fluorobenzyloxy)-benzylaminoproprionamide], Batch No. SG10859/47, were provided by Pharmacia, Nerviano, Italy, and were dissolved in distilled water. All solutions (saline was used as vehicle) were injected subcutaneously in a volume of 2 ml/kg, s.c.. Dosages are expressed as the free base. The dose of MPTP applied has not been found to affect food/water intake excessively. However, in all the experiments special precautions are taken to facilitate each animal´s ability to acquire food/water by placing each appropriately on the floor of the cage for the first two days following the MPTP treatment.

Chronic administration of L-Dopa

Groups of mice (n = 12 mice in each group) were injected s.c. with either MPTP (2 × 40 mg/kg, 24 hrs separating each injection) or saline (control) eight-to-nine weeks before the behavioural testing.

Tolerance-to-L-Dopa procedure

Three weeks after MPTP/saline treatment, groups of MPTP-treated and saline-treated mice were assigned to the L-Dopa-tolerance condition and these mice were administered L-Dopa (20 mg/kg, s.c.) everyday for five days each week, mon.-fri., weekends were injection-free [as outlined in the chapter by Archer and Fredriksson, (1999)] until L-Dopa (20 mg/kg) had been administered on twenty-five occasions (total of five weeks). Motor activity parameters were tested during a 120-min period in the test chambers on two occasions only, i.e. the first and the twenty-fifth day of L-Dopa injection, as the step-by-step progressive nature of the tolerance development or "wearing-off" to these daily injections of L-Dopa has been reported previously (Fredriksson et al., 1999b). The mice were then tested from the week following onwards for the restorative experiments outlined below:

Groups of mice (n = 10 or 12) were administered with MPTP (six or eight groups in the case of each test compound), except for some minor modifications, as outlined previously (cf. Archer and Fredriksson, 1999). L-Dopa-tolerant MPTP mice were administered either CGP 40116 (0.01, 0.03 or 0.1 mg/kg, s.c.), memantine (0.1, 0.3, 1, 3 or 10 mg/kg, s.c.), amantadine (0.3, 1, 3, 10 or 30 mg/kg, s.c.), MK-801 (0.01, 0.03, 0.1, 0.3, or 1 mg/kg, s.c.), lamotrigine (1.0, 3.0 or 10.0 mg/kg, s.c.), FCE 26743 (1.0, 3.0 or 10.0 mg/kg, s.c.) or saline (vehicle) 60 min before placement in the activity test chambers. 60 min after placement in the test chambers each mouse was injected L-Dopa (20 mg/kg) or saline and then replaced in its test chamber again. Motor activity parameters were then registered over a total test period of 180 min.

Neurochemical analysis

Mice were killed by cervical dislocation within two weeks of completion of behavioural testing. Determination of DA was performed using an high-performance liquid chromatograph with electrochemical detection (HPLC-EC), according to (Björk et al., 1991), as modified (Liu, 1995). Striatal regions were rapidly dissected out and stored at −80°C until neurochemical analysis. DA concentration was measured as follows: The frozen tissue samples were weighed and homogenized in 1 ml of 0.1 M perchloric acid, and alpha-methyl-5-hydroxytryptophan was added as an internal standard. After centrifugation (12,000 rpm, i.e. 18,600 g, 4°C, 10 min) and filtration, 20 μl of the supernatant was injected into the HPLC-EC to assay DA. The HPLC system consisted of a PM-48 pump (Bioanalytical Systems, BAS) with a CMA/240 autoinjector (injection volume: 20 μl), a precolumn (15 × 3.2 mm, RP-18 Newguard, 7 μm), a column (100 × 4.6 mm, SPHERI-5, RP-18, 5 μm), and an amperometric detector (LC-4B, BAS, equipped with an Ag/AgCl reference electrode and a MF-2000 cell) operating at a potential of +0.85 V. The mobile phase, ph 2.69, consisted of K2HPO4 and citric acid buffer (pH 2.5), 10% methanol, sodium octyl sulphate, 40 mg/l, and EDTA. The flow rate was 1 ml/min, and the temperature of the mobile phase was 35°C.

Statistical analysis

The locomotion, rearing and total activity data for the total test period (180 min) from each of the experiments where each single-drug administration was combined with L-Dopa (chronic 20 mg/kg) were submitted to a one-way ANOVA, based on a completely randomised design (Kirk, 1995). Pairwise testing between the different treatment groups was performed with the Tukey HSD test (ibid). The 1% level of significance was maintained throughout, unless where otherwise stated.

Results

Chronic suprathreshold dose of L-Dopa

Effect of repeated administration of a threshold dose of L-Dopa: L-Dopa (20 mg/kg) produced a straightforward reinstatement, in MPTP-treated mice, of basal levels of motor activity encompassing locomotion, rearing and total activity during Days 1 to 13 (and to a lesser extent Day 14) of drug administration during the second hour after L-Dopa injection. The enhanced motor activity in MPTP mice induced by L-DOPA showed a step-wise dimunition from administration Day 15 onwards and reaching base level (comparable to MPTP mice not administered L-Dopa) on Days 18 to 20 and onwards (Fredriksson et al., 1999). An increase in the basal levels of motor activity was observed for the control mice, throughout. All the MPTP mice administered L-Dopa repeatedly to induce tolerance (or "wearing-off") demonstrated a marked reduction [step-by-step (15)] of post-administration motor activity parameters from Day 1 of L-Dopa injection to Day 25 (see Table 2). By Day 25, all the MPTP-treated demonstrated baseline (non-drug) responses to L-Dopa treatment. In the case of CGP 40116 and MK-801, MPTP mice were administered L-Dopa over 22 days and were tested for the restorative action of the glutamate antagonists on Days 23–27 (with L-Dopa injections continued), whereas for all other compounds they were tested Days 26–30.

Table 1. Effects of repeated administration of L-Dopa (20 mg/kg, s.c.) upon motor behaviour of MPTP-treated and control mice. Locomotion, rearing and total activity counts of MPTP-treated mice administered repeated injections of L-Dopa (20 mg/kg) five times each week over five weeks: there was a drastic reduction of the motor activity response to the compound from Day 1 of injection to Day 25. MPTP treatment (2 × 40 mg/kg, s.c.) was administered four to six weeks before testing. L-Dopa administration was performed as described previously (Archer and Fredriksson, 1999)

Counts per 2-hr period

Group	Locomotion	Rearing	Total Activity
Control[1]	1,886 ± 358	1,025 ± 228	9,911 ± 3,112
Day 1:sal	239 ± 143	203 ± 99	3,194 ± 1,267
Day 1:L-D	977 ± 279	588 ± 217	7,117 ± 1417
Day 25:sal	169 ± 112	131 ± 90	3,498 ± 984
Day 25:L-D	253 ± 101*	167 ± 91*	3,732 ± 1014*
[%]	[26]	[28]	[52]

Values are expressed as means ± SD of 12 (control) or 96 (MPTP) mice.
* $p < 0.01$, versus Day 1:L-D, Student's t-test. [%] = Day 25:L-D counts expressed as a percentage of Day 1:L-D counts.
[1] Saline-treated mice tested on Day 1 after saline, injected saline throughout and tested following saline injection on the Test day (Saline + Saline).

The effectiveness of combining various non-competitive and competitive glutamate antagonistic compounds with L-Dopa (20 mg/kg) for inducing a *restoration* of motor activity in L-Dopa-tolerant, hypokinetic MPTP mice were as follows:

Effect of CGP 40116

Pretreatment with CGP 40116 (0.01 or 0.03 mg/kg, immediately before placement in the activity test chambers) restored the antiakinesic actions of L-Dopa (20 mg/kg) in tolerance-developed mice, with a magnitude that was notably greater than that shown by the mice injected MK-801 that was administered as reference compound to CGP 40116 in this experiment (see Table 2). One-way ANOVA comprising the three CGP40116 dose groups, a vehicle-L-Dopa group and a Vehicle-saline group for both MPTP and control mice indicated significant Between-Groups effects: [n = 10] $F(9, 90) = 29.93$, $F(9, 90) = 19.69$, and 16.88 for Locomotion, Rearing and Total activity, respectively. Figure 1 presents the mean locomotion, rearing and total activity counts during the 180-min test interval by CGP 40116 or vehicle plus L-Dopa or saline administered mice pretreated with either MPTP of saline (control).

Table 2. Locomotion, rearing and total activity by mice pretreated with MPTP (2 × 40 mg/kg, s.c., 24 hrs apart), and administered L-Dopa (20 mg/kg, s.c.) or saline chronically over 22 days and were tested on Days 23–27 (L-Dopa/saline injections continued). CGP 40116 (0.01, 0.03 or 0.1 mg/kg, s.c.) or MK-801 (0.03, 0.1 or 0.3 mg/kg, s.c.) in the Low (L), Medium (M) and High (H) dose groups, respectively, were injected immediately before placement in the motor activity test chambers, and L-Dopa was injected one hour later. The values are expressed as a quotient of the respective locomotion/rearing/total activity counts of each dose group divided by that of the counts made by the same group on Day 1 (first injection and activity with L-Dopa) and then multiplied by 100

Activity	Interval[1]	Sal-Sal	Sal-Dopa	MK(L)-Dopa	MK(M)-Dopa	MK(H)-Dopa
Locom.	120–180	123	17	20	84*	34
Rear.	-"-	100	13	40	34	38
Total act.	-"-	114	16	36	85*	39
Locom.	60–120	113	118	126	138	134
Rear.	-"-	116	63	85	60	69
Total act.	-"-	108	124	133	146	148
Activity	Interval[1]	Sal-Sal	Sal-Dopa	CGP(L)-Dopa	CGP(M)-Dopa	CGP(H)-Dopa
Locom.	120–180	146	19	92*	135*	32
Rear.	-"-	100	21	82*	116*	29
Total act.	-"-	103	23	94*	151*	36
Locom.	60–120	124	129	139	158	137
Rear.	-"-	111	50	106	109	64
Total act.	-"-	109	135	149	175	136

Locom., Rear., and *Total act.* Locomotion, Rearing and Total activity, respectively.
[1] refers to the interval following placement in the test chambers. 60–120 min was the period directly following L-Dopa injections.
* Significant degree of restoration on the L-Dopa motor activity effect.

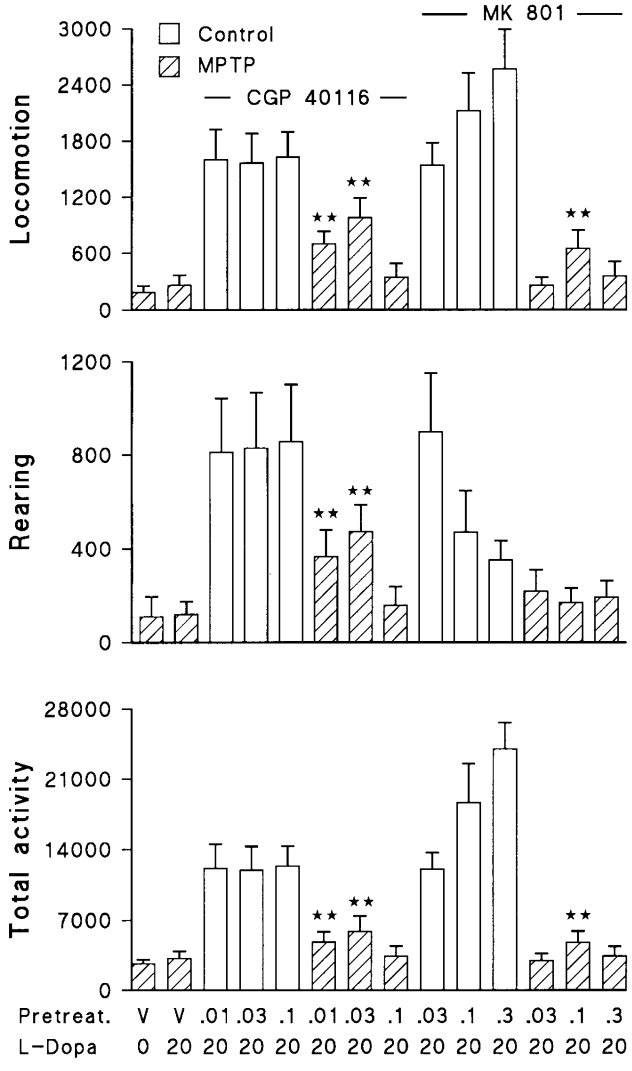

Fig. 1. Effects of the competitive and noncompetitive NMDA antagonists, CGP 40116 and MK-801, respectively, co-administered with a threshold dose of L-Dopa (20 mg/kg), upon motor activity by L-Dopa-tolerant MPTP-treated mice tested on Days 23–27 of L-Dopa administration. Locomotion, rearing and total activity counts of groups of mice administered either CGP 40116 (0.01, 0.03 or 0.1 mg/kg, s.c.), or MK-801 (0.03, 0.1 or 0.3 mg/kg, s.c.) or vehicle immediately prior to placement in the activity test chambers. L-Dopa (20 mg/kg) or saline were injected 60 min later and each mouse was replaced in its test chamber. Animals were tested over a total test period of 120 min. MPTP (2×40 mg/kg, s.c., 24-hr interval between injections) was injected three weeks before the start of the procedure for L-Dopa tolerance development, as assessed by L-Dopa-induced motor behaviour stimulaion, and approximately seven-and-a-half weeks before the testing of the NMDA-antagonists. *V*, Vehicle, *0*, saline, otherwise L-Dopa or antagonist dose. Values represent means ± SD of n = 12 mice. * versus Vehicle-L-Dopa, Tukey HSD-testing, $p < 0.01$

Tukey HSD-testing indicated significantly more locomotion, rearing and total activity counts by the 0.01 and 0.03 mg/kg dose groups compared with both vehicle MPTP groups at comparable levels to controls.

Effect of memantine

The co-administration of memantine with a suprathreshold dose (20 mg/kg) of L-Dopa to L-Dopa-tolerant MPTP mice elevated both locomotor (1 and 3 mg/kg) and rearing (0.3 and 3 mg/kg) behaviour at the specified doses, although a restoration was obtained for locomotor activity only at the 1 mg/kg dose. In the control mice, co-administration of memantine (1, 3 and 10 mg/kg) with L-Dopa dose-dependently increased locomotor behaviour and decreased rearing behaviour. Thus, one-way ANOVA indicated significant between-groups effects for both locomotion: $F(11, 132) = 118.15$, $p < 0.0001$, and rearing: $F(11, 132) = 54.65$, $p < 0.0001$. Figure 2 presents the locomotion and rearing counts of L-Dopa-tolerant MPTP and control mice co-administered L-Dopa with different doses of either memantine, amantadine or MK-801 or saline. Tukey HSD tests indicated the following pairwise differences:

In the MPTP-treated mice, the memantine (1 and 3 mg/kg) group performed significantly more locomotion counts than the saline + L-Dopa group whereas the memantine (0.3 and 3 mg/kg) + L-Dopa group performed significantly more rearing counts than the respective saline + L-Dopa group. In the control mice, the higher dose memantine (1, 3 and 10 mg/kg) + L-Dopa dose-dependently increased locomotor activity whereas the same groups dose-dependently decreased rearing activity. A complete/partial restorative effect of the test compound, for each given motor activity parameter, may be obtained by comparison of the drug-histograms with the broken line (see Fig. 2, left-hand panel).

Effect of amantadine

The co-administration of amantadine with a suprathreshold dose (20 mg/kg) of L-Dopa to L-Dopa-tolerant MPTP mice restored both locomotor (3 and 10 mg/kg) and rearing (3 mg/kg) behaviour at the specified doses. At the 30 mg/kg dose locomotor behaviour was enhanced. No effects of co-administration of the compound with L-Dopa in control mice were evident. Thus, one-way ANOVA indicated significant

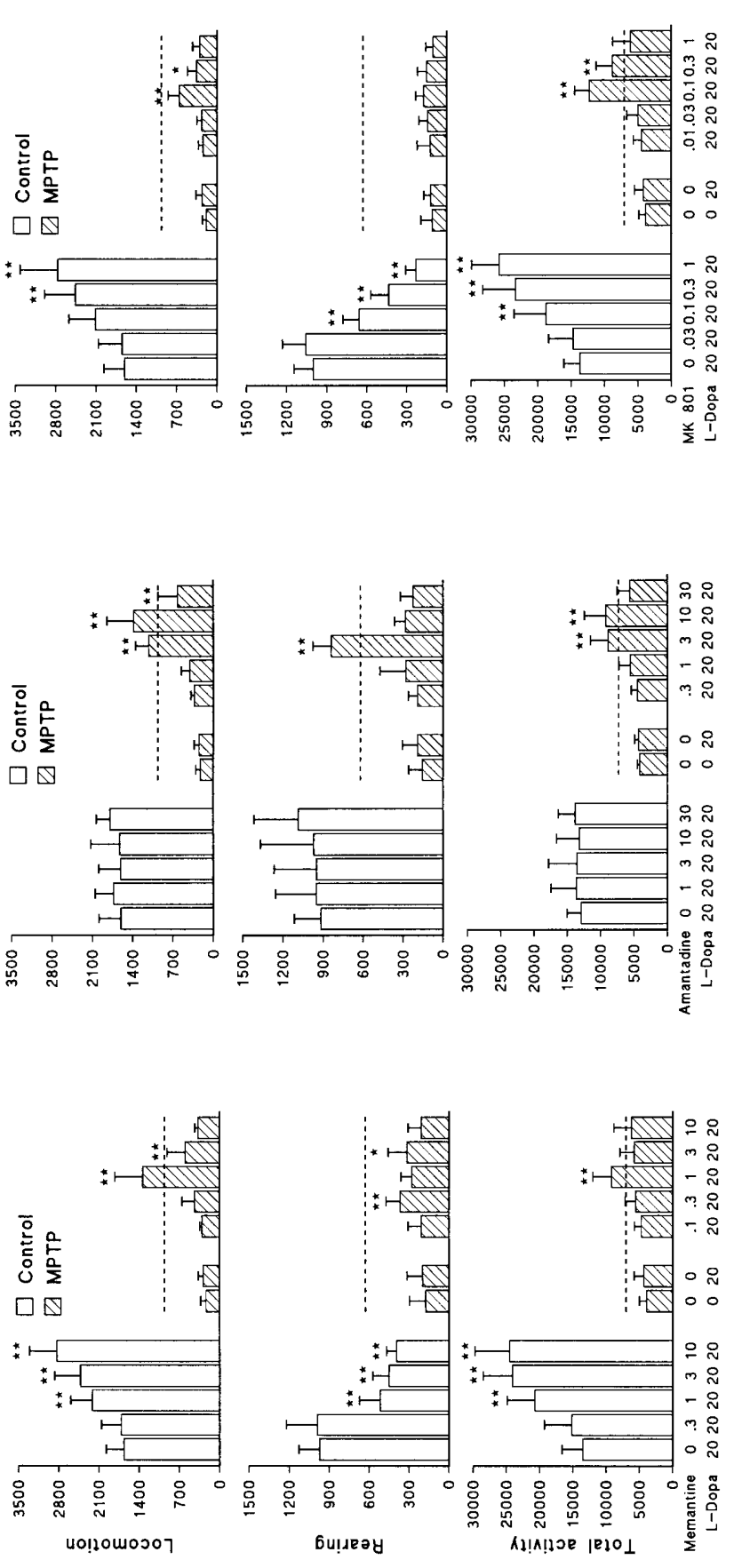

Fig. 2. Effects of the NMDA antagonists, memantine, amantadine and MK-801, co-administered with a threshold dose of L-Dopa (20 mg/kg), upon motor activity by L-Dopa-tolerant MPTP-treated mice. Locomotion and rearing counts by MPTP-treated and control mice administered memantine (0.1, 0.3, 1, 3 or 10 mg/kg, s.c.), amantadine (0.3, 1, 3, 10 or 30 mg/kg, s.c.) or MK-801 (0.01, 0.03, 0.1, 0.3 or 1 mg/kg, s.c.) or saline (6 groups) 60 min before placement in the test chambers. L-Dopa (20 mg/kg, s.c.) or saline was injected immediately before placement in the activity test chambers and the start of activity recording. Control mice received memantine (0.3, 1, 3 or 10 mg/kg), amantadine (1, 3, 10 or 30 mg/kg) or MK-801 (0.03, 0.1, 0.3 or 1 mg/kg) or saline 60 min before L-Dopa. MPTP (2×40 mg/kg, 24 hrs interval) was administered twice about nine weeks before testing. Each mouse was tested over a total accumulated period of 180 min, as represented by the histograms on the graph. The broken line represents mean motor activity counts by MPTP-treated mice injected saline chronically and tested with L-Dopa (20 mg/kg) under the same conditions (i.e. the extent of restoration). Values represent means ± SD of 12 mice. $^{**}p < 0.01$, $^{*}p < 0.05$, Tukey HSD tests

between-groups effects for both locomotion: F(11, 132) = 52.77, $p < 0.0001$, and rearing: F(11, 132) = 36.07, $p < 0.0001$. In the MPTP-treated mice, the amantadine (3, 10 and 30 mg/kg) + L-Dopa groups performed significantly more locomotion counts than the saline + L-Dopa group whereas the amantadine (3 mg/kg) + L-Dopa group performed significantly more rearing counts than the respective saline + L-Dopa group. No effects of amantadine were evident in the control mice. A complete/partial restorative effect of the test compound, for each given motor activity parameter, may be obtained by comparison of the drug-histograms with the broken line (see Fig. 2, middle panel).

Effect of MK-801

The co-administration of MK-801 with a suprathreshold dose (20 mg/kg) of L-Dopa to L-Dopa-tolerant MPTP mice elevated locomotor (0.1 and 0.3 mg/kg) but not rearing behaviour at the specified doses. In the control mice, MK-801 (0.3 and 1 mg/kg) increased locomotor and decreased (0.1 mg/kg dose also) rearing behaviour. Thus, one-way ANOVA indicated significant Between-Groups effects for both Locomotion: F(11, 132) = 35.79, $p < 0.0001$, and rearing: F(11, 132) = 24.10, $p < 0.0001$. In the MPTP-treated mice, the MK-801 (0.1 and 0.3 mg/kg) + L-Dopa group performed significantly more locomotion counts than the saline + L-Dopa group whereas there were no restorative effects of MK-801 on rearing behavior. In control mice, for locomotion: MK-801 (0.3 and 1 mg/kg) + L-Dopa > saline + L-Dopa, and for rearing: MK-801 (0.1, 0.3 and 1 mg/kg) + L-Dopa < saline + L-Dopa. A complete/partial restorative effect, relatively, of the test compound, for each given motor activity parameter, may be obtained by comparison of the drug-histograms with the broken line (see Fig. 2, right-hand panel).

Effect of lamotrigine

Lamotrigine, at the higher dose of 3.0 mg/kg, restored all three parameters of motor activity, locomotion, rearing and total activity, in co-administration with L-Dopa (20 mg/kg). The highest dose, 10.0 mg/kg, of lamotrigine restored also rearing behaviour. One-way ANOVA indicated significant Between-Groups effects for Locomotion: F(4, 45) = 16.81, Rearing: F(4, 45) = 29.73, and Total activity (4, 45) = 12.97. Tukey HSD-testing indicated significantly more locomotor and total activity counts at the 3.0 mg/kg dose, and for rearing at the 3.0 and 10.0 mg/kg doses (see Fig. 3, left-hand panel). Figure 3 presents the locomotion and rearing counts of L-Dopa-tolerant MPTP and control mice co-administered L-Dopa with different doses of either lamotrigine, FCE 26743 or MK-801 or saline.

Effect of FCE 26743

The putative antiepileptic compound, FCE 26743, in combination with L-Dopa, augmented motor responses in L-Dopa-tolerant MPTP-treated mice, increasing locomotion and total activity at the higher, 3.0 and 10.0 mg/kg doses, and rearing at the 1.0 and 3.0 mg/kg doses. One-way ANOVA indicated significant Between-Groups effects for Locomotion: F(4, 45) = 21.22, Rearing: F(4, 45) = 18.77, and Total activity: F(4, 45) = 13.28. Tukey HSD-testing indicated that the 3.0 and 10.0 mg/kg dose groups induced more locomotor counts than the group injected saline + L-Dopa; rearing counts were elevated by the 1.0 and 3.0 mg/kg dose groups (see Figure 3, middle panel). MK-801 affected the motor behaviour of both L-Dopa-tolerant MPTP-treated and saline-treated (control) mice (see Figure 3, right-hand panel), whereas the anticonvulsive agents, lamtrigine and FCE 26743, affected only the motor behaviour in response to acute L-Dopa in the hypokinesic MPTP mice.

Neurochemical analysis

MPTP-treated, L-Dopa-tolerant mice tested in co-administrations of the above compounds + L-Dopa showed severe depletions of DA in the striatum in comparison with drug-naive (repeated saline injections, saline-treated mice (see Table 3).

Discussion

"Wearing-off" fluctuations in MPTP mice chronically administered L-Dopa (20 mg/kg) over 22 or 25 days before testing, were shown by the loss of L-Dopa efficacy in elevating parameters of motor behaviour. The present results pertaining to effects of different doses of compound with glutamate antagonistic actions co-administered with suprathreshold L-Dopa to L-Dopa-tolerant MPTP-treated mice (there is no evidence that control mice developed a tolerance to the DA precursor) may be summarised as follows:

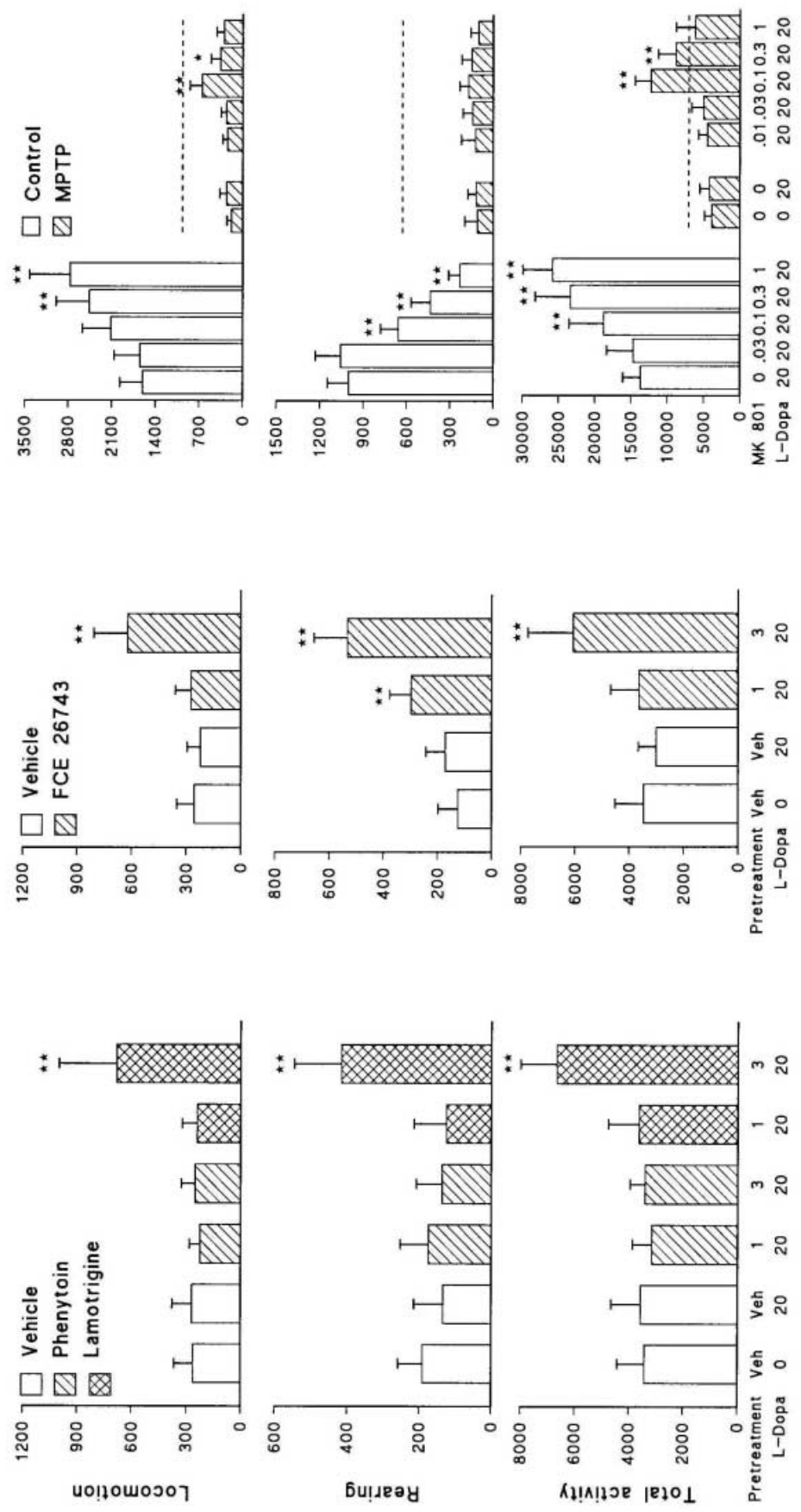

Fig. 3. Effects of the anticonvulsive agents and NMDA antagonists, lamotrigine and FCE26743, compared with the noncompetitive antagonist, MK-801, co-administered with a threshold dose of L-Dopa (20 mg/kg), upon motor activity by L-Dopa-tolerant MPTP-treated mice. Locomotion and rearing counts by MPTP-treated and control mice administered lamotrigine (1.0, 3.0, 10.0 mg/kg, s.c.), FCE 26743 (1, 3 or 10 mg/kg, s.c.) or MK-801 (0.01, 0.03, 0.1, 0.3 or 1 mg/kg, s.c.) or saline (6 groups) 60 min before placement in the test chambers. L-Dopa (20 mg/kg, s.c.) or saline was injected immediately before placement in the activity test chambers and the start of activity recording. Control mice received lamotrigine (1.0, 3.0, 10.0 mg/kg, s.c.), FCE 26743 (1, 3 or 10 mg/kg, s.c.) or MK-801 (0.03, 0.1, 0.3 or 1 mg/kg) or saline 60 min before L-Dopa. MPTP (2×40 mg/kg, 24 hrs interval) was administered twice about nine weeks before testing. Each mouse was tested over a total accumulated period of 180 min, as represented by the histograms on the graph. The broken line represents mean motor activity counts by MPTP-treated mice injected saline chronically and tested with L-Dopa (20 mg/kg) under the same conditions (i.e. the extent of restoration). Values represent means ± SD of n = 12 mice. $^{**}p < 0.01$, $^{*}p < 0.05$, Tukey HSD tests; *Veh*, vehicle

Table 3. Striatal dopamine (DA) concentrations following treatment with either MPTP (2 × 40 mg/kg, s.c.), administered on two occasions separated by a 24-hr interval, of mice tested in the chronic, suprathreshold L-Dopa dose tests, or mice injected saline throughout the period of chronic administration and then injected saline at testing (Saline + Saline). MPTP-treated L-Dopa-tolerant mice were randomly assigned to neurochemical determination. The mice were killed by cervical dislocation one week after completion of behavioural testing. Determinations of DA were performed using an high-performance liquid chromatograph with electrochemical detection (HPLC-EC)

	MPTP	Saline
Dopamine (μg/g wet weight of tissue) (%)	4.09 ± 0.75*	19.14 ± 0.95 (21)

Values represent means ± s.e.m. of 6 mice; * $p < 0.01$, Student's t-test; (%) = percent of control values.

In experiments employing the suprathreshold dose of L-Dopa (20 mg/kg) in the L-Dopa-tolerant mice procedure: (1) In MPTP-treated mice displaying "wearing-off", CGP 40116 co-administered with L-Dopa restored dose-dependently the L-Dopa-induced motor activity whereas MK-801 restored dose-dependently those functional effects of the precursor compound. The effects of the former upon locomotion, rearing and total activity were of a greater order of magnitude than that of the latter. At these doses, CGP 40116 did not exert any behavioural effects on either L-Dopa-treated or saline-treated control mice. (2) Amantadine (locomotion: 3, 10 and 30, rearing: 3 mg/kg) and memantine (locomotion: 1 and 3, rearing: 0.3 and 3 mg/kg) either completely or partially restored (see broken lines on Figures 4 and 5) the antihypokinesic effects of the suprathreshold dose (20 mg/kg) of L-Dopa to which tolerance or "wearing-off" had been completed over four weeks of chronic administration. (3) MK-801 (0.1 and 0.3 mg/kg) in this specific test restored partially the antihypokinesic effects of L-Dopa with regard to locomotor activity only. (4) The anticonvulsant agent, lamotrigine, was effective in restoring L-Dopa-activity at the higher doses of 3.0 and 10.0 mg/kg over all three motor activity parameters whereas FCE 26743 was effective for locomotion and total activity at those doses, but at the 1.0 mg/kg dose as well for rearing in the L-Dopa-tolerant MPTP mice. (5) In the control mice, co-administration of memantine (1, 3 and 10 mg/kg) with L-Dopa dose-dependently increased locomotor behavior and decreased rearing behavior. MK-801 (0.3 and 1 mg/kg) increased locomotor activity and reduced rearing (0.1 mg/kg as well) behavior. (6) Neither lamotrigine, FCE 26743 nor CGP 40116 affected motor activity parameters in L-Dopa-chronically administered control mice. In each case a restorative effect of the test compounds (the NMDA antagonists) may be assessed by the extent to which each histogram-dose effect tangents the broken lines (see Fig. 2). Amantadine did not affect the behavior of control mice. Neurochemical analysis of DA concentrations in the striatum of L-Dopa-tolerant MPTP-treated mice indicated a marked depletion (79% loss of DA). Thus, the results of the experiments applying chronic treatment with suprathreshold L-Dopa (20 mg/kg) to produce a "wearing-off"of its efficacy, suggest a restoration, and in some cases a partial restorative, effect, following co-administration with specific doses of the NMDA antagonists (in comparison with an MPTP group chronically treated with saline and administered saline + L-Dopa at testing, see Figs. 4–6), roughly in the order of: CGP 40116 > MK-801, or amantadine > memantine > MK-801, or FCE 26743 = lamotrigine > MK-801.

The co-administration of the competitive glutamate antagonist, CGP 40116, with L-Dopa was found to induce a more than complete restoration of locomotor behaviour (135% of the L-Dopa effect shown on Day 1, see Table 2) at the most effective dose, 0.03 mg/kg, whereas an almost complete restoration (92% of the L-Dopa effect on Day 1, see Table 2) was obtained at the lowest dose, 0.01 mg/kg, and a marginal effect (32% of the L-Dopa effect compared with the 19% of the Sal-Dopa group) at the highest dose of 0.1 mg/kg. Rearing and total activity were restored also to comparable extents (Rearing: 116%, 82% and 29% for 0.03, 0.01 and 0.1 mg/kg doses, respectively, Total activity: 151%, 94% and 36% for 0.03, 0.01 and 0.1 mg/kg doses, respectively). In previous studies (Fredriksson et al., 1994a,b), the effects of single doses of either CGP 40116 or MK-801 in combination with a single, initial subthreshold dose of L-Dopa (5 mg/kg) upon parameters of motor activity were measured in MPTP-treated and control mice. It was shown that the co-administration of CGP 40116 and L-Dopa induced significant increases in locomotor and rearing behaviour over an impressively wide dose range (0.001 to 0.03 mg/kg) whereas MK-801 was effective only at the 0.1 mg/kg dose. Thus, the relative efficacies of CGP 40116 and MK-801, derived from the present chronic, suprathreshold-dose L-Dopa procedure, and the pre-

vious acute, subthreshold-dose procedure, would appear to be roughly comparable. Similarly, the relative efficacies of memantine, amantadine and MK-801, on the one hand, and lamotrigine and FCE 26743 (if say compared to an ineffective compound like phenytoin) on the other hand, regarding the chronic, suprathreshold versus acute, subthreshold L-Dopa procedures were roughly comparable (Fredriksson et al., 1999a, 2000).

The synergistic and restorative effect of the compounds are reflected against the above background of hypokinesis in MPTP mice. Nevertheless, although aspects of the conclusion pertain to the interpretation that the restoration of locomotion and/or rearing behaviour of MPTP mice by these compounds (in co-administration with either 5 mg/kg of 20 mg/kg L-Dopa) is specifically linked to antiparkinsonian and anti-"wearing-off" effects, respectively, some degree of caution is necessary. Thus, it should be indicated that no *direct* evidence is provided here for this interpretation unrestrictedly, particularly in the case of the memantine (Fig. 2) and MK-801 (Figs. 2 and 3) experiments, since the control animals also showed significant behavioural changes at the effective drug doses. On the other hand, the interpretations regarding the efficacy of amantadine for synergism and restoration seem undeniable.

Engber et al. (1994), utilising the unilateral 6-OHDA lesion rotational model in rats, demonstrated that the duration of contralateral rotation to acute L-Dopa injection was reduced by about 30% from Day 1 to Day 22 (L-Dopa administration: Day 1 duration of rotation = about 112 min, as estimated from figure). In the present study applied MPTP mice whose motor behaviour was measured in test chambers, locomotion, rearing and total activity counts were reduced by 86%, 81% and 85%, respectively, from Day 1 to Day 20 of L-Dopa administration (Absolute values for Day 1 of L-Dopa, 3rd hour, Locomotion: 587 counts, Rearing: 302 counts, and Total activity: 5727 counts). Those authors (ibid) obtained a near complete reinstatement (approximately 94% of L-Dopa group rotation on Day 1 compared to the 70% shown on Day 22 in the absence of MK-801; see Engber et al., 1994) of L-Dopa + carbidopa induced contralateral turning behaviour following the 0.1 mg/kg dose of MK-801. Contrastingly, the present results showed a less complete restoration of locomotor behaviour by MPTP mice that received a similar dose, 0.1 mg/kg, of MK-801 + L-Dopa (84% of the L-Dopa effect on Day 1, see Table) at testing on Day 23: this should be compared with the saline + L-Dopa group on the same day (17% of L-Dopa effect on Day 1, see Table 2). The highest dose of MK-801, 0.3 mg/kg, induced a much less complete degree of restoration (34% compared to the 17% of the saline + L-Dopa group, see Table 2). Some discrepancy is understandable in view of the parametric, pharmaceutical and procedural (L-Dopa + carbidopa vs L-Dopa alone, L-Dopa doses, number of drug administrations during induction of "wearing-off", daily administration schedule, interval between MK-801 and L-Dopa, comparisons over successive test days with same group rather than same test day and different groups, etc) and methodological (rats vs mice, unilateral intracerebral 6-OHDA vs systemic MPTP, rotational-monitoring apparatus vs ADEA activity test chambers, etc) differences between the two studies. For rearing behaviour, the combination of MK-801 with L-Dopa failed to restore motor activity significantly at any of the doses applied (0.03, 0.1 or 0.3 mg/kg, which gave 40%, 34% and 38%, respectively, of the L-Dopa effect on Day 1, see Table 2, but see also Figure 1); note that the rearing counts by the saline + L-Dopa group on the same day were also less (13% of L-Dopa effect on Day 1, see Table). The total activity results resembled those of locomotion in the extent of restoration (36%, 85% and 38% for the 0.03, 0.1 and 0.3 mg/kg doses, respectively, of the L-Dopa effect on Day 1, see Table 2).

The present results confirm much other evidence of the activity-enhancing effects of NMDA antagonists with L-Dopa: e.g. elevations of motor activity with in combinations of MK-801 with L-Dopa (Goodwin et al., 1992), or MK-801 with dihydrexidine (Gossel et al., 1995). Neither MK-801 nor CGP 40116 restored motor activity in MPTP mice when injected with saline. In DA-depleted MPTP-treated animals either drug-naive or after chronic administration of L-Dopa, competitive/noncompetitive NMDA antagonists together with L-Dopa induced synergistic effects on motor activity (cf. Archer et al., 1996). Several other investigations have documented synergistic effects of combining L-Dopa with NMDA antagonists but it should be noted that in these cases excessive concentrations of the DA-precursor were applied: Maj et al. (1993) showed that CGP 37849 (1.0 and 3.0 mg/kg), but not CGP 39551, plus L-Dopa (50 mg/kg, and benserazide, 100 mg/kg) caused a synergistic action on locomotor activity. Similarly, Greenamyre et al. (1994) found that the anticonvulsant agent, remacemide hydrochloride

(5–40 mg/kg, p.o.) co-administered with a subthreshold dose of L-Dopa methylester dose-dependently increased motor activity. Remacemide is also an uncompetitive NMDA antagonist with neuroprotective properties (Palmer et al., 1995). In parkinsonian rhesus monkeys, remacemide (10 mg/kg, p.o.) potentiated the effects of suprathreshold doses of L-Dopa methylester (100–200 mg/kg, i.p.). Such positive interactions between memantine or amantadine and L-DOPA have been reported in several animal models of Parkinsonism (Gossel et al., 1995) but the present findings offer synergistic-restorative actions at remarkably lower doses those used previously (above). Despite dose considerations, the ability of amantadine and memantine to potentiate the antiparkinson actions of L-Dopa in the clinic has been demonstrated (Rabey et al., 1992).

The mechanism of potentiation of L-Dopa action by NMDA antagonism might involve inhibition of overactive descending glutamatergic input from the cortex to striatum (Schmidt and Kretschmer, 1997) although other possible drug-neurotransmitter-receptor site interactions should be taken into account. Recently, Fisher et al. (1998) examined the acute effects of NMDA and non-NMDA antagonists on the activity of aromatic l-amino acid decarboxylase (AADC) in the striatum and substantia nigra of rats. MK-801 (0.01, 0.1 and 1.0 mg/kg) and phencyclidine (4 mg/kg) elevated AADC in both regions (2-to-3 fold), and even more so amantadine, 40 mg/kg (striatum: 3.8 fold; substantia nigra: 9.0 fold), and memantine, 40 (striatum: 3.4 fold; substantia nigra: 3.1 fold). Interestingly, CGP 40116 at 1 and 5 mg/kg, had no effect upon AADC activity but it should be noted that the compound affects L-Dopa responses in MPTP-treated mice at much lower doses, i.e. 0.01 and 0.03 mg/kg (Archer and Fredriksson, 1999). It was suggested that the ability of amantadine and memantine to potentiate the antiparkinson actions of L-Dopa may be due to facilitated decarboxylation of L-Dopa by the brain (Fisher et al., 1998). The novel aspect of such a potentially theraputic combination is believed to consist of an attenuation of the motor fluctuations, seen after prolonged exposure to L-Dopa, by administration of NMDA receptor antagonists (Bravi et al., 1994).

The antiglutamatergic properties of anticonvulsant agents like lamotrigine (as suggested by Jones-Humble et al., 1993) may offer this underlying mechanism as modulating the restorative effects upon motor functions of MPTP mice obtained (Archer and Fredriksson, 1999). For example, Zipp et al. (1993) examined the therapeutic benefits of lamotrigine (administered in combination with doses of L-Dopa ranging from 450 to 600 mg) in five PD patients. Of these, three showed clear improvements, one showed a transient improvement and the fifth patient no definite improvement. In the laboratory, felbamate, an anticonvulsive compound that induces an NMDA-like effect in inhibiting NMDA receptor responses (Rho et al., 1994) was shown to antagonise the D_2 (haloperidol) receptor-mediated, but not the D_1 (SCH 23390) receptor-mediated, cataleptic response (Kretschmer, 1994), an animal model of parkinsonism. Interestingly, Kaur and Starr (1996) examined the effects of combining lamotrigine (5–40 mg/kg, i.p.) with either L-Dopa (150 mg/kg, i.p.), the D_1 agonist, SKF 38393 (30 mg/kg, i.p.), or the D_2 agonist, RU 24213 (5 mg/kg, s.c.) upon motor behaviour of dopamine-depleted mice. It was found that lamotrigine (10 and 40 mg/kg) enhanced significantly the antiakinetic action of L-Dopa in the parkinsonian mice, possibly through a D_2, rather than D_1, mediation. Greenamyre et al. (1994) showed that the anticonvulsive agent, remacemide hydrochloride, (5–40 mg/kg, p.o.) co-administered with a subthreshold dose of L-Dopa methylester dose-dependently increased motor activity in parkinsonian rhesus monkeys. Remacemide (10 mg/kg, p.o.) potentiated the effects of suprathreshold doses of L-Dopa methylester (100–200 mg/kg) and similar to lamotrigine the antiparkinsonian effects were suggested to involve antiglutamatergic mediation, since remacemide is an uncompetitive NMDA antagonist (Palmer et al., 1995).

In the present study, all three compounds, amantadine, memantine and MK-801, partially or completely restored in a dose-related manner the antihypokinesic effect of the suprathreshold dose of L-Dopa following previous chronic administration ("wearing off"). This is in line with previous studies in rats with unilateral 6-hydroxydopamine lesions of the nigrostriatal system, showing that MK-801 and prolongs the duration of L-Dopa-induced contralateral rotations in rats chronically administered L-Dopa over three or four weeks (Engber et al., 1994; Papa et al., 1995). Similar effects were described for amantadine (50 mg/kg) (Karcz-Kubicha et al., 1998). Clinical studies confirm such positive effects for amantadine and memantine in L-Dopa tolerant patients (Rabey et al., 1992; Shannon et al., 1987). Interestingly, amantadine (and some

other agents having antagonistic effects on NMDA receptors) inhibits also the dyskinesias evoked by prolonged L-Dopa treatment both in MPTP-treated monkeys and in Parkinsonian patients (Blanchet et al., 1997; Verhagen Metman et al., 1998a,b). Hence, it has been suggested that both dyskinesias and "wearing-off" involve similar mechanisms, namely an oversensitivity of NMDA receptors (mainly NR1/NR2B) in the striatum due to enhanced phosphorylation, resulting from tonic activation of the DA receptors (Oh et al., 1998). Thus, as suggested recently (Danysz et al., 1997; Lange et al., 1997), the aminoadamantanes, memantine and amantadine, show an antiparkinsonian action in animal models and in Parkinsonian patients possibly through NMDA receptor blockade, although this mechanism remains less certain in the case of amantadine.

Out of all the agents tested, only amantadine is administered on regular basis for the treatment of Parkinson's disease. The first indications that the substance may possess an antiparkinsonian action date from the early 1970s when Strömberg and Svensson (1971) found that amantadine increased motor activity in mice and ipsilateral rotational behavior in unilaterally striatomized rats. These authors suggested that the antiparkinsonian action of amantadine could be explained by catecholamine release in the brain. Note however, that in those studies substantially greater concentrations of the compound were applied: certainly at those concentrations (50–150 mg/kg) catecholamine release may be expected. In the clinic, Blomberg et al. (1972) demonstrated that amantadine had an assured effect in two-thirds of PD cases. Hypokinesia and rigidity were most affected whereas tremors were reduced to a lesser extent. These studies confirmed the positive results of other studies (e.g. Hughes et al., 1971; Kannari and Markstein, 1991; Laitinen and Vilkki, 1971). However, the finding that amantadine inhibits both dyskinesias and "wearing off" effects in Parkinsonian patients following long lasting exposure to L-Dopa opens new avenues for therapeutic use of this "old agent", particularly at the dose levels used in the present study.

In conclusion, the putative role of the NMDA receptor antagonists, memantine and amantadine, as antiparkinsonian agents is suggested by their synergistic activity-enhancing actions upon co-administration with subthreshold L-Dopa to hypokinesic MPTP-treated mice. In light of the discovery of NMDA-receptor existence, cellular specificity and pharmacological diversity (Standaert et al., 1994), enhancement of the action of suprathreshold dose of L-Dopa following tolerance development renders a potentially restorative effect and suggests a useful application of these agents in the treatment of "wearing off" fluctuations in the clinic.

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