Differing Effects of N-methyl-D-aspartate Receptor Subtype Selective Antagonists on Dyskinesias in Levodopa-Treated 1-Methyl-4-phenyl-tetrahydropyridine Monkeys


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ABSTRACT

The antiparkinsonian and antidyskinetic profile of two N-methyl-D-aspartate (NMDA) receptor antagonists, a competitive antagonist, (R)-4-oxo-5-phosphononorvaline (MDL 100,453), and a novel noncompetitive allosteric site antagonist, 4-hydroxy-N-[2-[(4-hydroxyphenoxy)ethyl]-4-(4-methylbenzyl)piperidine (Co 101244/PD 174494), was assessed in six levodopa-treated 1-methyl-4-phenyl-tetrahydropyridine-lesioned parkinsonian monkeys. The effects on motor function of these two drugs, alone and in combination with levodopa, were then correlated with NMDA subtype selectivity and apparent affinity for four diheteromeric NMDA receptor subunit combinations expressed in Xenopus oocytes. MDL 100,453 (300 mg/kg s.c.) by itself increased global motor activity (p < .0005 versus vehicle) and administered 15 min after a low dose of levodopa/benserazide s.c., MDL 100,453 (50, 300 mg/kg s.c.) showed dose-dependent potentiation of antiparkinsonian responses and also produced dyskinesias. Following injection of a fully effective dose of levodopa, MDL 100,453 (300 mg/kg s.c.) also produced a 25% increase in mean dyskinesia score (p = .04). In contrast, Co 101244 did not change motor activity by itself and only showed a tendency to potentiate the antiparkinsonian response when given in combination with a low dose of levodopa, which did not attain statistical significance. However, with a high dose of levodopa, Co 101244 (0.1, 1 mg/kg s.c.) displayed antidyskinetic effects (67 and 71% reduction, respectively) while sparing levodopa motor benefit. In vitro, MDL 100,453 was an NMDA glutamate-site antagonist, with ~5- to 10-fold selectivity for the NR1α/NR2A subtype combination (Kᵢₑᵣ = 0.6 μM) versus NR1α in combination with 2B, 2C, or 2D. In contrast, the allosteric site antagonist Co 101244 showed ~10,000-fold selectivity for the NR1α/NR2B (IC₅₀ = 0.026 μM) versus the other three subunit combinations tested. Taken together, the data suggest that the NR2 subunit selectivity profile of NMDA receptor antagonists can play an important role in predicting behavioral outcome and offer more evidence that NR2B-selective NMDA receptor antagonists may be useful agents in the treatment of Parkinson’s disease.

Oral levodopa replacement therapy remains the single most effective medication for the symptomatic relief of Parkinson’s disease (PD). However, predictable and unpredictable fluctuations in clinical status and abnormal involuntary movements (dyskinesias) eventually develop in most patients following chronic levodopa treatment (Marsden, 1990). Recent pharmacological evidence suggests that levodopa-related motor response complications in experimental parkinsonism in rats are due, at least in part, to hyperfunctioning of certain central glutamatergic pathways (Engber et al., 1994; Oh et al., 1997, 1998). In support of this, a variety of N-methyl-D-aspartate (NMDA) receptor antagonists have been shown to be effective in animal models of PD. For example, in 1-methyl-4-phenyl-tetrahydropyridine (MPTP)-lesioned parkinsonian monkeys treated with levodopa, a competitive NMDA receptor glutamate-site antagonist (LY 235959) significantly attenuated dyskinesias while sparing the motor benefit derived from levodopa (Papa and Chase, 1996). The therapeutic index of this novel agent was far superior than that of the potent NMDA channel blocker MK-801 (Crossman et al., 1989; Close et al., 1990; Rupniak et al., 1992; Domino and Sheng, 1993) and appreciably better than the weak channel blocker amantadine (Blanchet et al., 1998).

Mammalian NMDA receptors are ligand-gated ion channels composed of di- or triheterooligomeric assemblies of NR1

ABBREVIATIONS: PD, Parkinson’s disease; Co 101244/PD 174494, 4-hydroxy-N-[2-[(4-hydroxyphenoxy)ethyl]-4-(4-methylbenzyl)piperidine; DSI, dyskinesia severity index; MDL 100,453, (R)-4-oxo-5-phosphononorvaline; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NMDA, N-methyl-D-aspartate; PAM, primate activity monitor.
subunits and NR2 subunits (Moriyoshi et al., 1991; Hollmann and Heinemann, 1994). Individual NR1 isoforms and NR2 subunits have distinct anatomical and developmental patterns of expression (Sheng et al., 1994). In addition, different subunit combinations (or “subtypes”) have distinct biophysical and pharmacological characteristics (Williams, 1993; Hollmann and Heinemann, 1994; Priestly et al., 1995; Woodward et al., 1995). Overall, the molecular biology studies give strong support to the idea that different NMDA subtypes mediate different aspects of brain function, and that the subtype-selectivity profile of NMDA antagonists will affect their therapeutic potential and side effect profile.

The key pharmacological properties of NMDA receptor antagonists important for the treatment of PD remain poorly understood. Having an optimum pattern of subtype selectivity is only the beginning. Other variables are certainly important, for example: 1) site/mechanism of antagonist action (e.g., channel blockers, competitive antagonists, allosteric inhibitors etc.), 2) affinity/binding kinetics, and 3) efficacy of inhibition (i.e., full versus partial). Comparison of activity in vivo with mechanism and subtype selectivity in vitro should yield important insights into structure-function relationships in NMDA responses as well as improved therapies for PD. In view of the antidyskinetic effect reported earlier with the competitive glutamate-site antagonist LY235959 (Papa and Chase, 1996), we evaluated another competitive glutamate-site antagonist (R)-4-oxo-5-phosphononorvaline (MDL 100,453) and a novel allosteric NMDA antagonist 4-hydroxy-N-[2-(4-hydroxyphenoxy)ethyl]-4-(4-methylbenzyl)piperidine (Co 101244/PD 174494) for antiparkinsonian and antidyskinetic efficacy in monkeys. We also assayed these compounds, together with LY 235959, for inhibitor potency and subtype selectivity at four cloned binary NMDA subtypes expressed in Xenopus oocytes (NR1A or NR1E in combination with NR2A, NR2B, NR2C, or NR2D). The results indicate that different NMDA receptor antagonists can have opposite effects on motor function and that subtype selectivity may play a role in determining these differences.

Materials and Methods

Animal Study Design. Six cynomolgus (Macaca fascicularis) monkeys of both sexes (2 females and 4 males) weighing 3.25 to 7.4 kg were studied under an approved protocol that met the ethical standards of the institutional Animal Care and Use Committee. Subjects were housed individually, fed with a standard biscuit diet twice a day supplemented with fruits, and had free access to water. They were kept under stable room conditions and maintained under a 12-h light/dark cycle. All monkeys were exposed to MPTP hydrochloride (Research Biochemicals Intl., Natick, MA) administered s.c. at a weekly dose of 0.5 to 1 mg/kg until sustained parkinsonian features with action tremor appeared. The average cumulative MPTP dose was 4.4 mg/kg (range, 2.1 to 9.75 mg/kg). All animals were left drug-free for 6 to 8 weeks and scored on a regular basis using the Laval University Disability Scale for MPTP Monkeys (Gothen et al., 1998). Briefly, the monkeys were moved to an observation room and watched for 20 min for habituation and determination of a reliable baseline rating. Once injected, they were maintained under direct observation to be rated along the Laval University Disability Scale for MPTP Monkeys (Gomez-Mancilla et al., 1993) by the same blinded investigator (S.K.) every 15 min until a complete return to baseline. A definite antiparkinsonian response was considered present as long as the baseline motor subscore was improved by at least 2 points. The magnitude of the motor response was given by the absolute number of points of improvement accrued over 60 min after the NMDA antagonist was administered. Dyskinesias were scored along a simple Abnormal Involuntary Movement Scale (Blanchet et al., 1998) and a dyskinesia severity index (DSI) calculated using the formula: (sum of all dyskinesia scores/duration of antiparkinsonian effect determined by direct observation) × 100. A continuous assessment of the total motor activity reflecting objective changes over baseline status was also obtained from a primate activity monitor (PAM) tied underneath a primate jacket and providing motor counts every 2 min. The same PAM was always used for a given animal and retrieved under sedation once the whole protocol was completed. Motor counts were accrued over 4 h following a drug injection as a measure of the total drug effect on general motor behavior influenced by but not discriminating dyskinetic movements from spontaneous activity.

Statistical analysis was conducted with SigmaStat version 2.03 software (SPSS Inc., Chicago, IL). The duration of the response and total PAM activity accrued over 4 h were pooled and differences assessed with a repeated measures ANOVA followed by Dunnnett’s post hoc test. Behavioral ratings (magnitude of the response and DSI values) resulting from the different treatments were compared with Friedman ANOVA followed by Dunnnett’s post hoc test. A p value <.05 was considered statistically significant.

Electrophysiology. cDNA clones encoding the rat NMDA receptor subunits NR1A, NR2A, NR2B, NR2C, and NR2D were provided by Dr. P. H. Seeburg (Heidelberg University, Heidelberg, Germany; Monyer et al., 1992). The NR1 eDNA was provided by Dr. S. Nakahashi (Kyoto University, Kyoto, Japan). Preparation and maintenance of Xenopus laevis oocytes and microinjection with cRNA were all as previously described (Woodward et al., 1995; Whittemore et al.,...
Effects of MDL 100,453 and Co 101244 on cloned NMDA receptors were assayed by measuring inhibition of membrane current responses elicited by fixed concentrations of the coagonists glutamate and glycine. Both agonists were applied at saturating, or near saturating, concentrations: 10 μM glycine plus 100 μM glutamate for NR1A/2A; 1 μM glycine plus 100 μM glutamate for NR1A/2A, NR1A/2B, NR1A/2C, and NR1A/2D. Oocytes were exposed to agonists until a steady-state current was obtained, and then superfused with a conventional flow-through chamber (volume ~0.2 ml). Under these conditions, provided levels of expression were moderate, coapplication of glutamate and glycine elicited a monophasic NMDA response (Whittemore et al., 1997).

The predetermined low near-threshold dose of levodopa, averaging 32 ± 10 mg (range, 2.5–60 mg), significantly increased PAM counts by 50% (Table 1) but produced no detectable change in motor ratings given the sensitivity of our scale and inclusion of several other motor parameters. In combination with the low dose of levodopa, MDL 100,453 (300 mg/kg) potentiated PAM activity (p = .01) and antiparkinsonian efficacy in all subjects (Table 1). By comparison, Co 101244 produced inconsistent potentiation effects. With the low dose of levodopa, Co 101244 (1 mg/kg) only showed potentiation in half the subjects, sufficient to improve average PAM counts and to lower disability scores (Table 1).

The predetemined high dose of levodopa averaged 63 ± 20 mg (range, 15–150 mg). The dose fully reversed parkinsonian disability for an average duration of 82 min and increased PAM counts by 138% (Table 1). Neither MDL 100,453 nor Co 101244 altered this response profile when combined with the high dose of levodopa (Table 1).

The high dose of levodopa produced choreic dyskinasias in four animals and predominantly dystonic dyskinasias in two animals for a combined mean DSI value of 20 ± 4 (range, 7.5–26). No change in DSI was seen after administration of the low dose of MDL 100,453, but a significant 25% increase...
was documented when levodopa was combined with MDL 100,453 (300 mg/kg; Fig. 1). In contrast, Co 101244 significantly decreased mean DSI values by 67 and 71% following doses of 0.1 and 1 mg/kg, respectively (Fig. 1). Both chorea and dystonia subscores were improved. In view of the magnitude of the antidyskinetic effect resulting from Co 101244, we elected to raise the levodopa dose further in five subjects to a mean of 172 mg (range, 40–500 mg), for a mean DSI value of 25, and tested it under the same conditions. Co 101244 still displayed good antidyskinetic activity with reductions in mean DSI values of 56 and 62% following doses of 0.1 and 1 mg/kg, respectively, without impacting on motor benefit (duration, magnitude, or PAM activity; data not shown).

Inhibition of Cloned NMDA Receptors Expressed in Oocytes. The previously tested NMDA receptor glutamate-site antagonist LY 235959 inhibited agonist-evoked currents with IC50 values between 0.25 and 0.6 μM, dependent on the subunit combination (Fig. 2 and Table 2). The calculated Kd values demonstrate that the apparent affinity of LY 235959 for different NMDA receptor subunit combinations varies by only 3-fold across the five subunit combinations tested. The competitive, glutamate-site antagonist MDL 100,453 inhibited currents with IC50 values ranging between 3.6 and 36 μM depending on the subunit combination (Fig. 2 and Table 2). Converting IC50 values for individual subunit combinations into Kd values demonstrated that, in terms of apparent affinity, MDL 100,453 had 5- to 10-fold selectivity for the NR1α/2A versus other subunit combinations (Fig. 2 and Table 2). In contrast, the allosteric site antagonist Co 101244 inhibited agonist-evoked currents at NR1α/2B and NR1ε/2B with IC50 values of 0.024 and 0.048 μM, respectively, but was essentially inactive at NR1α/2A, NR1α/2C, and NR1α/2D (Fig. 2 and Table 2). Thus, Co 101244 showed ~10,000-fold selectivity for the NR1α/2B subunit combination (Zhou et al., 1998).

Discussion

The two NMDA antagonists assayed in the present experiments, MDL 100,453 and Co 101244, had distinct, and to a large extent opposite behavioral effects in the MPTP-treated monkeys. The two compounds differ in terms of their mechanism of antagonism: MDL 100,453 is a competitive antagonist, whereas Co 101244 is an allosteric inhibitor. The electrophysiological measurements showed that the two compounds also have profoundly different subtype-selectivity profiles for inhibition of binary NMDA receptor subunit combinations expressed in oocytes. Most strikingly, MDL 100,453 showed ~5-fold greater potency for NR1α/NR2A (Kd = 0.6 μM) versus NR1α/2B (Kd = 3.2 μM), whereas Co 101244 potently inhibited the NR1α/2B (IC50 = 0.0026 μM) and was essentially inactive at other NR2 subtype combinations (IC50 > 200 μM). Combining the NR2B subunit with NR1ε, an NR1 isoform that contains the C-terminal insert cassette (Zukin and Bennett, 1995), did not affect the potency of either compound. This is consistent with previous studies indicating that the potency of competitive and allosteric antagonists shows dependence on the NR2 subunit and not the NR1 isoform (Lynch and Gallagher, 1996; Gallagher et al., 1996; Fischer et al., 1997).

Given the distinct localization of NMDA receptor subtypes in mammalian brain (Monyer et al., 1994; Sheng et al., 1994; Zukin and Bennett, 1995), it is not surprising that MDL 100,453 and Co 101244 produced widely different behavioral effects. Less easy to explain are the behavioral differences seen between MDL 100,453 and the previously tested competitive antagonist LY 235959 (Papa and Chase, 1996). The present experiments indicate that LY 235959 is essentially nonelective across the four binary NMDA subunit combinations, with potencies varying only ~2-fold (Kd = 0.04–0.09 μM). Again, incorporation of NR1ε did not affect potency, at least for NR2B-containing receptors. Thus, LY 235959 has a subtype profile and mechanism of inhibition closer to MDL 100,453 than Co 101244 and yet, in terms of behaviors, it
more closely resembles Co 101244. Reasons for the apparent disparity between subtype profile and antiparkinsonian and antidyskinetic activity are uncertain. It is possible that the 

\[ \text{NR2A/2B receptors, which are considered to be a major brain subtype (Luo et al., 1997).} \]

Unfortunately, assaying drugs on triheteromeric receptors in oocytes is problematic because it is difficult to distinguish between populations of true triheteromeric subunit combinations and mixtures of tri- and diheteromeric combinations, all of which form functional receptors (Stocca and Vicini, 1998). When extrapolating from work with the clones to complex behavioral models, it is also important to appreciate the numerous other factors that could come into play in vivo. These include differences in binding kinetics between antagonists, competition with and modulation by endogenous agents, and interactions at other NMDA subtypes or at other classes of receptor/channel. Such differences are certainly worth investigating to compare the antiparkinsonian efficacy of nonselective NMDA antagonists in monotherapy, like amantadine and MDL 100,453, to NR2B-selective NMDA antagonists like CP-101,606 (Steece-Collier et al., 1995) and ifenprodil (Nash et al., 1997) that have shown some efficacy in preliminary studies conducted in primates. The foregoing results and others (Papa and Chase, 1996) on the efficacy of NMDA antagonists cast doubt on the superiority of NR2B-selective antagonists to improve mobility in monotherapy. Comparative studies between NR2A- and NR2B-selective NMDA antagonists would be worth pursuing.

The two antagonists also differed in their interaction with exogenous levodopa. MDL 100,453 consistently potentiated levodopa responses, as reported previously with other NMDA blockers (Löschmann et al., 1991; Wüllner et al., 1992; Greenamyre et al., 1994): the highest dose turned a near-threshold low dose of levodopa into an antiparkinsonian and dyskinetic dose, and increased the dyskinetic scores following a high dose of levodopa. In contrast, Co 101244 less consistently potentiated the motor response to levodopa and produced a good antidyskinetic effect similar to the competitive NMDA glutamate-site antagonist LY 235959 (Papa and Chase, 1996). Just how certain NMDA antagonists potentiate levodopa-related benefit whereas others attenuate dyskinesia remains unexplained, but because both Co 101244 and LY 235959 displayed higher affinity for the NR1A/NR2B combination than MDL 100,453, this in itself may well be a feature for attenuation of dyskinesias.

The differences in affinity and selectivity found between the two antagonists tested for subtypes of NMDA receptors are likely to be linked to different mechanisms of action and circuits in the basal ganglia. Although NMDA receptors are widely expressed in the brain (Monyer et al., 1992; Buller et al., 1994), previous work indicates that intrastriatal administration of the NMDA channel blocker MK-801 reverses response alterations recorded in 6-hydroxydopamine-lesioned rats following chronic levodopa treatment (Papa et al., 1995). Moreover, a similar effect following the intrastriatal injection of a protein kinase A inhibitor (Oh et al., 1997) and a tyrosine kinase inhibitor (Oh et al., 1998) has been reported in the same model, the latter drug also attenuating the enhanced tyrosine phosphorylation of striatal NMDA NR2A and NR2B subunits observed following chronic levodopa treatment (Oh et al., 1998). This clearly suggests a striatal site of action. Nonetheless, pharmacological blockade of extrastriatal glutamate pathways may also affect basal ganglia outflow and in doing so promote antiakinetic and prodyskinetic effects, as seen following local perfusion of kynurenic acid.
acid (broad spectrum glutamate antagonist) in the internal pallidal segment (GPI) in normal monkeys (Robertson et al., 1989). Thus, subthalamo-pallidal (GPI) mechanisms could conceivably play a greater role in the case of MDL 100,453 compared with Co 101244 to enhance dyskinesia. In contrast, Co 101244 could antagonize glutamate influences at the level of the external pallidal segment (GPe), where local application of kynurenic acid did not provoke dyskinesia (Robertson et al., 1989). Interestingly, injections of kynurenic acid in the ventral part of the GPe in awake monkeys provoked contralateral leg dystonia (Robertson et al., 1989) and high doses of the NMDA antagonist MK-801 (Rupniak et al., 1992) and LY 235959 (Papa and Chase, 1996) have increased dystonia severity. In one PD patient off levodopa, some doses of the noncompetitive NMDA receptor channel blocker dextorophan apparently worsened foot dystonia, whereas it attenuated peak-dose chorea in combination with levodopa (Blanchet et al., 1996). Thus, direct and/or indirect modulation of neuronal activity in subregions of the GPe may partly explain the antydyskinetic activity of certain NMDA blockers.

The foregoing results provide insights for the development of NMDA antagonists with optimal pharmacological properties for treating the disabling and prevalent condition of levodopa-induced dyskinesia. Antagonists showing selectivity for receptors containing the NR2B subunit may be particularly efficacious in this respect. Whether the antydyskinetic effects of NMDA antagonists will be maintained with chronic treatment and whether these drugs can slow the onset of dyskinesias following early combination with levodopa remain important questions well worth pursuing.

Acknowledgments

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References


### Table 2

<table>
<thead>
<tr>
<th>Drug/Receptor Subunits</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; μM</th>
<th>Slope</th>
<th>K&lt;sub&gt;B&lt;/sub&gt; (Leff-Dougall)</th>
<th>Ocytes Tested</th>
</tr>
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<tbody>
<tr>
<td>LY235959</td>
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<tr>
<td>NR 1ε/2B</td>
<td>0.30 ± 0.03</td>
<td>1.0 ± 0.09</td>
<td>0.05</td>
<td>3</td>
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<tr>
<td>NR 1ε/2A</td>
<td>0.60 ± 0.02</td>
<td>1.2 ± 0.04</td>
<td>0.1</td>
<td>1</td>
</tr>
<tr>
<td>MDL 100,453</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NR 1ε/2A</td>
<td>3.6 ± 0.4</td>
<td>1.0 ± 0.12</td>
<td>0.60</td>
<td>3</td>
</tr>
<tr>
<td>NR 1ε/2B</td>
<td>17 ± 1.7</td>
<td>1.3 ± 0.15</td>
<td>3.21</td>
<td>4</td>
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<tr>
<td>NR 1ε/2C</td>
<td>36 ± 1.7</td>
<td>1.2 ± 0.06</td>
<td>4.35</td>
<td>3</td>
</tr>
<tr>
<td>NR 1ε/2D</td>
<td>17 ± 1.0</td>
<td>1.2 ± 0.09</td>
<td>5.77</td>
<td>3</td>
</tr>
<tr>
<td>NR 1ε/2B</td>
<td>19 ± 2.0</td>
<td>1.4 ± 0.19</td>
<td>3.53</td>
<td>1</td>
</tr>
</tbody>
</table>

All experiments were performed in nominally Ca<sup>2+</sup>-free frog Ringers, with Ba<sup>2+</sup> in place of Ca<sup>2+</sup>. Glycine was fixed at 1 μM for NR 1ε/2B, NR 1ε/2B, and NR 1ε/2C, and at 10 μM for NR 1ε/2A.


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