Published in final edited form as:

Ann Neurol. 2004 November ; 56(5): 723–727. doi:10.1002/ana.20279.

Prolongation of Levodopa Responses by Glycine_B Antagonists in Parkinsonian Primates

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Abstract

To examine the antiparkinsonian effects of blocking glycine_B receptors, we designed a pilot study testing the potent and selective antagonist, PAMQX, in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated primates. PAMQX had no intrinsic effects but markedly potentiated the antiparkinsonian action of levodopa. In a dose-dependent fashion, coadministration of the glycine_B antagonist with levodopa extended the response duration by nearly 60%. It is noteworthy that PAMQX, within a considerable dose range, did not cause ataxia or other side effects. These data indicate that blocking *N*-methyl-D-aspartate receptors selectively to manipulate dopaminergic-mediated motor responses may be produced effectively by glycine_B antagonists.

In Parkinson's disease, nigrostriatal dopamine denervation leads to basal ganglia functional alterations that are largely mediated by glutamate transmission. Likewise, pharmacological manipulation of glutamatergic activity has proved effective in various animal models, particularly by the antiparkinsonian action of *N*-methyl-D-aspartate (NMDA) antagonists.^{1–3} However, the therapeutic potential of these agents often declines as a result of significant toxicity.^{4–7} Noncompetitive and competitive NMDA antagonists have a narrow therapeutic window because most effective doses produce marked adverse reactions such as psychiatric symptoms, ataxia, and sedation (anesthetic-like effects).

Heteromeric NMDA receptors require the binding of glutamate and glycine for channel activation. As opposed to glutamate-site antagonists, those selective for the glycine site (glycine_B) lack adverse psychotomimetic effects but still maintain anticonvulsant or neuroprotective properties.^{8,9} Thus, glycine_B antagonists possess a different functional profile that might result in similar motor effects with lower toxicity, which is a critical aspect for therapeutic applications. On the basis of the foregoing premise, we examined the antiparkinsonian effects of a full, short-acting, and highly selective glycine_B antagonist, PAMQX,^{10,11} in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)–primate model of Parkinson's disease.

Materials and Methods

Subject Preparation

Three adult monkeys (*Macaca mulatta*) were housed and studied in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. They previously had been infused through the internal carotid artery¹² with 0.4mg/kg of MPTP. At the time of this study, they exhibited a chronic hemiparkinsonism and had been drug free for several months. We predetermined two doses of subcutaneous L-dopa methyl ester plus benserazide (Sigma-Aldrich, St. Louis, MO): “optimal,” the minimal dose that produced maximal effects (LD-high, 100–200mg), and “suboptimal,” the dose that produced considerably lower but unequivocally measurable effects (LD-low, 50–100mg).

Experimental Drug Tests

1-PAMQX DOSE-RESPONSE CURVE—In set 1, the vehicle or one of four doses of PAMQX (Novartis Pharma AG, Basel, Switzerland) was injected first, and saline (L-dopa vehicle) was injected subsequently. In set 2, the vehicle was injected first and one of the two doses of L-dopa was injected afterward. In these experiments, motor disability and balance and stability were evaluated (see following). Doses of PAMQX were 1, 2, 4, and 10mg/kg.

2-PAMQX/L-DOPA COADMINISTRATION WITH ASSESSMENT OF MOTOR DISABILITY AND BALANCE AND STABILITY—For combination with L-dopa, we selected the two middle-range doses of PAMQX (2 and 4mg/kg), because the highest dose was used as an overload to evaluate toxicity. Tests started with LD-low combined with PAMQX 2 or 4mg/kg. Subsequently, only the dose of PAMQX that had an effect was combined with LD-high to determine whether the optimal L-dopa effect could be augmented. LD-high was combined only with PAMQX 4mg/kg. These treatments were compared with LD-low and LD-high from the previous set.

3-PAMQX/L-DOPA COADMINISTRATION WITH ASSESSMENT OF A MOTOR TASK—The same combinations of treatments as above were administered. LD-low and LD-high were repeated for assessment of the motor task.

Motor disability was assessed with a standardized scale for MPTP-treated primates, as previously described.⁴ Balance and stability were assessed with the “climbing test” that consisted of climbing several perches in a rod or ladder to reach the ceiling of the cage (scale: body swinging: none 0, mild = 1, moderate = 2, severe = 3; tilting: none = 0, mild = 1, moderate = 2, severe = 3. Number of a: lapses, and b: falls). The motor task consisted of picking up food using a Klüever board (action time and index of success were measured).

All tests were performed after overnight fast, and drugs were given subcutaneously. Animals were videotaped for subsequent rating by a blinded investigator. Each test in all sets of experiments was repeated. Data were averaged to yield a mean from three data points for each treatment in each monkey.

Statistical Analysis

Data are presented as mean $\pm$ standard error of the mean scores for each time interval. Two-factor repeated measures analysis of variance, followed by the post hoc Fisher’s Protected least significant difference (PLSD) test when the *f* indicated significance, were used to compare every treatment over the time course after drug administration.

Results

PAMQX Dose–Response Curve

PAMQX had no intrinsic antiparkinsonian effects. Motor disability scores averaged between 9.33 ± 0.3 and 10 ± 0.6 after vehicle and all PAMQX doses. PAMQX did not induce ataxia or other adverse effects on motor behavior. Animals scored zero (normal) in the climbing test at most examinations in all experiments. Monkeys did not exhibit retching, vomiting, or changes in social interaction.

LD-high nearly reversed parkinsonian symptoms, although monkeys still scored a mild degree of disability. LD-low clearly had lower effects (Fig, panels A, B). Responses to both doses began at between 15 and 20 minutes and peaked at from 30 to 50 minutes after the injection; duration of the “on” state was 60 to 80 and 80 to 100 minutes after LD-low and LD-high, respectively. Responses could not be extended by administering higher doses of L-dopa (data not shown).

PAMQX and L-Dopa Coadministration

PAMQX markedly potentiated the antiparkinsonian effects of L-dopa. Coadministration of 4mg/kg of PAMQX with the suboptimal dose of L-dopa produced a larger on response than that of L-dopa alone (see Fig, A). This difference derives from a tendency to increase the peak effect and, more consistently, from a pronounced prolongation of response duration. LD-low + PAMQX-4mg/kg effects were significantly different from baseline (vehicle) until 110 minutes. This effect was 40 minutes longer than the LD-Low Effect, which was significantly different from baseline only for 70 minutes (see Fig, A). Beyond 110 minutes, LD-low + PAMQX-4mg/kg still had a tendency to maintain a reduced score. The combination LD-low + PAMQX-4mg/kg resulted in similar but yet slightly longer antiparkinsonian effects than those of LD-high (see Fig, B). Overall, the potentiation of L-dopa action is fully expressed by comparing the global effect of each treatment. The total percentage of change from the “off” state produced by LD-low + PAMQX-4mg/kg doubles that of LD-low (see Fig, C). The addition of PAMQX to the optimal dose of L-dopa (LD-high + PAMQX-4mg/kg) did not produce significant benefit, although a trend for prolongation of the on state was evident (see Fig, B). PAMQX effects were similar in all monkeys, with prolongation of the on state between 40 and 50 minutes by LD-low + PAMQX-4mg/kg. Lower doses of PAMQX (LD-low + PAMQX-2mg/kg) were ineffective (see Fig, D).

Balance and stability were unaffected (climbing test). Here again, animals scored zero at most examinations in all experiments.

Action times in the motor task were markedly slower in the impaired hand ($p < 0.05$, paired t test, normal vs impaired hand). The addition of PAMQX to L-dopa had a tendency to sustain faster action times over longer periods in the impaired hand. This was indicated by a significant f for interaction in analysis of variance for repeated measures with nonsignificant effect for individual intervals in post hoc tests. These results can be attributed to group heterogeneity resulting from the varied severity of a single symptom in a low number of subjects.

Discussion

Our results show that glycine_B receptor blockade has major antiparkinsonian effects without apparent toxicity on motor or cognitive behavior. PAMQX had no effects per se, but it markedly potentiated L-dopa action by prolonging the response duration by nearly 60%. This is particularly important to help reducing L-dopa doses in patients with motor complications

associated with long-term therapy. As an antecedent, NMDA channel blockade was reported to reverse motor fluctuations in fully denervated rodents.¹³ However, synergistic effects of NMDA antagonists in primates usually are seen as augmentation of the peak effect^{3,5} instead of as the robust prolongation of the on state induced by PAMQX. Presumably, this effect also occurs with parent drugs and could be detected if responses of effective doses were not masked by side effects. We used from 1 to 10mg/kg of the glycine_B antagonist and did not find sedation or other common adverse effects, even under our thorough screening for ataxia. Thus, conclusions of this pilot study are, first, that glutamate antagonists can extend the antiparkinsonian action of L-dopa, and second, that blockade of the glycine site of NMDA receptors may be the best choice to develop antagonists of sufficient efficacy for clinical trials.

PAMQX, a very polar and soluble compound as most of its class, does not undergo enzymatic metabolism, and thus, there is no interaction with L-dopa pharmacokinetics. PAMQX effects on L-dopa responses derive from receptor coupling-related events. The glycine coagonist binding site is located in the NR1 subunit,¹⁴ whereas the glutamate binding site is in the NR2 subunit. The particular assembly of NR1, NR2, and NR3 subunits forms receptors with different binding affinity for glycine.¹⁵ Similarly, the affinity of antagonistic drugs for their recognition site depends on subunit composition. Therefore, compositional changes result in subpopulation of receptors with distinctive functional and pharmacological profiles. NMDA/glutamate site and glycine_B antagonists acting selectively on receptor subpopulations thus may produce markedly different effects. Further studies directed at characterizing the pharmacological profile of receptor subpopulations are needed to address the differential effects produced by PAMQX. In addition, extension of behavioral testing to models of motor complications is required to determine PAMQX effects on L-dopa-induced dyskinesias.

Previous studies of glycine_B antagonists in rodent models reported contradictory results.^{16,17} In those trials, long-acting antagonists and partial agonists were tested. Contrarily, PAMQX is a highly potent and selective antagonist. Its affinity for the glycine site is 600-fold to 2,000-fold higher than for NMDA and α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) glutamate sites, as shown by IC₅₀ in binding assays.^{10,11} In addition, dissociation rates are crucial to determine receptor-mediated responses, and PAMQX is a short-acting antagonist (see results from anticonvulsant protection tests^{10,11}). Full antagonists also differ in their ability to induce glycine-sensitive desensitization¹⁸ and, thereby, in their effect on the time course of receptor activation.⁸ Overall, several pharmacodynamic factors contribute to different effects of selective glycine_B antagonists. These results support the future role of glycine_B antagonists in the therapy of Parkinson's disease. PAMQX-like agents, especially short-acting and orally active drugs, need to be evaluated extensively for synergistic effects to dopaminergic agonists.

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Fig.

Effects of coadministration of PAMQX with L-dopa. (A) Each curve represents the effect of each treatment, vehicle (PAMQX vehicle + L-dopa vehicle), LD-low (PAMQX vehicle + LD-low), and LD-low + P-4mg/kg (LD-low + PAMQX-4mg/kg). Each data point is the mean score of motor disability from all monkeys ($n = 3$). Data of the off state correspond to baseline scores obtained just before drug injections (time 0); after injections scoring starts at 30 minutes and follows thereafter every 20 minutes. $*p < 0.05$ for both treatments versus vehicle. $**p < 0.05$ for differences between LD-low + P-4mg/kg versus vehicle and LD-low. Note that at these intervals LD-low is no longer different than vehicle. (B) As above, each curve represents the effect of each treatment, vehicle (PAMQX vehicle + L-dopa vehicle), LD-high (PAMQX vehicle + LD-high), and LD-high + P-4mg/kg (LD-high + PAMQX-4mg/kg). Significant differences between treatments and baseline were omitted. (C) Curves represent the percentage of change from the off score of motor disability for each

treatment. Each smoothed data point in the curves is the mean from all monkeys. Error bars were omitted for clarity. Areas under the curve are vehicle, 334 ± 120 ; LD-low, $2,338 \pm 203$; LD-low + P-4mg/kg, $4,835 \pm 363$. $p < 0.005$ for LD-low versus vehicle, and $p < 0.001$ for LD-low + P-4mg/kg versus vehicle and LD-low. (D) Comparison between the two doses of PAMQX demonstrates the dose-dependent effect. As above, each curve represents the effect of each treatment. Here, the treatment LD-low + P-2mg/kg (LD-low + PAMQX 2mg/kg) is included. $**p < 0.05$ for differences between LD-low + P-4mg/kg versus all other treatments. The duration of the on state is represented by horizontal lines for LD-low and LD-low + P-4mg/kg, as denoted by their patterns.