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Neurotrophic actions of the novel AMPA receptor potentiator, LY404187, in rodent models of Parkinson's disease

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Abstract

Recent developments in the molecular biology and pharmacology of α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors has led to the discovery of selective, potent and systemically active AMPA receptor potentiators. These molecules enhance synaptic transmission and evidence suggests that they play important roles in plasticity and cognitive processes. Activation of AMPA receptors also increases neuronal activation and activity-dependent signalling, which may increase brain-derived neurotrophic factor (BDNF) expression and enhance cell proliferation in the brain. We therefore hypothesised that an AMPA receptor potentiator may provide neurotrophic effects in rodent models of Parkinson's disease. In the present studies we report that the potent and selective AMPA receptor potentiator, *R*,*S*-*N*-2-(4-(4-Cyanophenyl)phenyl)propyl 2-propanesulfonamide (LY404187), provides both functional, neurochemical and histological protection against unilateral infusion of 6-hydroxydopamine into the substantia nigra or striatum of rats. The compound also reduced 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced toxicity in mice. Interestingly, we were also able to observe large functional and histological effects when we delayed treatment until after cell death had occurred (3 or 6 days after 6-hydroxydopamine infusion), supporting a neurotrophic mechanism of action. In addition, LY404187 provided a dose-dependent increase in growth-associated protein-43 expression in the striatum. Therefore, we propose that AMPA receptor potentiators offer the potential of a new therapy to halt the progression and perhaps repair the degeneration in Parkinson's disease.

Keywords: Parkinson's disease; Neuroprotection; AMPA receptor potentiator; LY404187; 6-Hydroxydopamine; MPTP; GAP-43 (growth-associated protein-43); BDNF (brain-derived neurotrophic factor); (Rat)

1. Introduction

Parkinson's disease is a progressive, neurodegenerative disorder of the basal ganglia (O'Neill and Siemers, 2002). The most striking pathological change in the parkinsonian brain is a loss of dopaminergic cells in the substantia nigra (SN), which project to the terminal rich caudate and putamen of the corpus striatum (Hornykiewicz and Kish, 1987). Clinical symptoms are manifested when approximately 60% of cell bodies have degenerated and initial symptoms of Parkinson's disease include tremor at rest, muscular rigidity, bradykinesia, postural abnormalities and instability (Hughes et al., 1992). The available pharmacotherapies to treat Parkinson's disease may be broadly viewed as dopamine replacement strategies and while these drugs reduce symptom severity they do not dramatically affect disease progression. Within 10–15 years of the initial diagnosis the typical prognosis is complete akinesia and often dementia (O'Neill and Siemers, 2002). Therefore, in order to maintain an acceptable quality of life for patients with Parkinson's disease, therapies that slow or stop disease progression are greatly needed.

The direct application of neurotoxins such as 6-hydroxydopamine (6-OHDA) into either the cell bodies and terminal fields of the nigrostriatal pathway has been used to model the neurodegeneration of this pathway that is a hallmark of Parkinson's disease (Zigmond and Stricker, 1989; O'Neill and Siemers, 2002). A variety of growth factors, including glial-derived neurotrophic factor (GNDF) and brain-derived

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neurotrophic factor (BDNF), have been reported to promote the survival of dopaminergic neurons in culture (Nakajima et al., 2001) and protect against neurotoxin-induced lesions of the nigrostriatal system (Altar et al., 1992; Gash et al., 1996; Rosenbald et al., 2000). A major drawback of growth factors is the need for central application. We hypothesized that a small molecule capable of stimulating the production of one or more of these growth factors would be useful in the treatment of Parkinson's disease.

It has been known for more than a decade that AMPA receptor activation produces a robust increase in BDNF expression in primary neuron cell culture (Zafra et al., 1990). Recently, a series of potent, selective and systemically active AMPA receptor potentiators have been described (Ornstein et al., 2000). These molecules (exemplified by *R*,*S*-*N*-2-(4-(3-Thienyl)phenyl)propyl 2-propanesulfonamide or LY392098 and *R*,*S*-*N*-2-(4-(4-Cyanophenyl)phenyl)-propyl 2-propanesulfonamide or LY404187) do not by themselves activate AMPA receptors, but markedly potentiate the effects of the endogenous transmitter, glutamate, or exogenously applied AMPA and are at least one order of magnitude more potent than the prototypical AMPA receptor potentiator, cyclothiazide (Gates et al., 2001; Miu et al., 2001). These compounds have recently been reported to increase the expression of BDNF in both primary neuron culture (Legutko et al., 2001) and in vivo (Mackowiak et al., 2000). It has also been demonstrated that LY404187 can produce discrete increases in glucose utilization in the rat brain (Fowler et al., 2002) and, more recently, the active isomer (LY451646) was shown to increase cell proliferation in the dentate gyrus (Bai et al., 2003).

To explore the hypothesis that a small molecule neuronal activator and stimulator of growth factor production would be effective in the treatment of Parkinson's disease, we evaluated LY404187 in rodent models of Parkinson's disease. The results of a series of studies indicated that LY404187 reduced 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced neurotoxicity in mice and provided functional, neurochemical and histological improvement after infusion of 6-hydroxydopamine into the substantia nigra or striatum of the rat. The functional and protective actions were associated with an increase in growth-associated protein 43 (GAP-43) expression in the striatum and were present when dosing was initiated 6 days after 6-hydroxydopamine infusion. Thus, AMPA receptor potentiators may provide a means of both halting the progression and perhaps reversing the degeneration characteristic of Parkinson's disease.

2. Methods

2.1. MPTP neurotoxicity in mice

Male C57BL/6J mice (Harlan UK, Oxford, UK) weighing 20–25 g were used. They were housed in groups of five mice per cage under a 12:12-h light/dark cycle (lights on 7:00 a.m. to 7:00 p.m.) with food and water available ad libitum. LY404187 was administered at 0.5 mg/kg s.c for 11 days. On day 8 the animals received 4×20 mg/kg MPTP at 2 h intervals.

2.2. 6-Hydroxydopamine studies in rats

Male Sprague–Dawley rats (Harlan UK) weighing 280–320 g were used. They were housed in groups of five rats per cage under a 12:12-h light/dark cycle (lights on 7:00 a.m. to 7:00 p.m.) with food and water available ad libitum. Rats were anaesthetised with a gaseous anaesthetic consisting of Isoflurane®/nitrous oxide/oxygen, were placed on a thermostatically controlled heating blanket and then placed in a Kopf stereotaxic frame. The scalp incised so as to uncover the parietal bones for drilling.

2.2.1. Striatal lesions

Unilateral lesions of the right striatum were made using 10 μg (free base) 6-hydroxydopamine in 2.57 μl 0.02% ascorbic acid in 0.9% saline infused stereotaxically into the right striatum—coordinates from bregma were AP: 0.7 mm, L: −2.3 mm, V: −6.0 mm (from skull surface at bregma) and toothbar −3.3 mm (Paxinos and Watson, 1986). The infusion was made over a period of 4 min at a rate of 0.643 μl/min, followed by 4 min equilibration time, with the cannula remaining in place. Sham-operated rats received identical surgery to the 6-hydroxydopamine group but 2.57 μl 0.02% ascorbic acid (6-hydroxydopamine vehicle) was infused. Thirty minutes prior to 6-hydroxydopamine or vehicle infusion, rats were pretreated with desipramine (12.5 mg/kg i.p.) to block uptake of the toxin into noradrenergic cells and pargyline (75 mg/kg i.p.) to block metabolism of the toxin.

2.2.2. Substantia nigra lesions

Unilateral lesions of the left substantia nigra were made using 4 μg (free base) 6-hydroxydopamine in 1.8 μl 0.02% ascorbic acid in 0.9% saline infused stereotaxically into the left substantia nigra—coordinates from bregma according to the atlas of Paxinos and Watson (1986) were AP: −4.8 mm, L: +1.9 mm, V: −8.0 mm (from skull surface at bregma) and toothbar −3.7 mm. 6-Hydroxydopamine was infused with a syringe infusion pump connected to a 28-gauge steel cannula. The infusion was made over a period of 6 min at a rate of 0.3 μl/min, followed by 2 min equilibration time, with the cannula remaining in place. Sham-operated rats received identical surgery to the 6-hydroxydopamine group but 1.8 μl 0.02% ascorbic acid (6-hydroxydopamine vehicle) was infused. Thirty minutes prior to 6-hydroxydopamine or vehicle infusion, rats were pretreated with desipramine (12.5 mg/kg i.p.) and pargyline (75 mg/kg i.p.).

2.2.3. Drug studies

For all studies, LY404187 was dissolved in 12.5% β-cyclodextrin and sonicated prior to administration and was administered subcutaneously (s.c.) twice daily on weekdays and once daily at weekends.

For studies using the striatal lesion model, LY404187 was administered for 28 days at 0.5 mg/kg s.c. starting 1 day after 6-hydroxydopamine lesion.

For nigral lesion studies, LY404187 was administered for 14 days at either 0.1 or 0.5 mg/kg s.c. starting 1 day after 6-hydroxydopamine lesion. In additional studies treatment with LY404187 was delayed until 3, or 6 days after 6-hydroxydopamine infusion.

2.3. Behavioural assessment using rotometers

Twenty-four hours after the final drug treatment, the animals were placed in automated rotometers (Med. Associates). The apparatus consisted of Perspex bowls where each rat was linked to a harness that had an infrared sensor at the top connected to a computer with ROTORAT software. This software measured the number of contraversive and ipsiversive turns (rotations). The animals were tested for baseline rotations 24 h after the final drug treatment. On the next day, the animals were retested in the presence of apomorphine (0.25 mg/kg s.c.) to evaluate the effects of drug treatment on stimulant-induced rotations (this is a measure of functional neuroprotection). Data was expressed as mean asymmetry scores (the difference between the number of contraversive and ipsiversive rotations).

2.4. Neurochemical measurements of dopamine and metabolites

The left and right striata were dissected, weighed and homogenised in two volumes of distilled water. A 10-μl aliquot of the homogenate was transferred to a 0.5-ml Eppendorf tube and 20 μl of 1% aqueous trifluoroacetic acid added, mixed and spun at 13,000 rpm for 5 min. Two microliters of the supernatant was then assayed by high-performance liquid chromatography with electrochemical detection. All analyses were performed on a Luna 5 C18 column (25 cm × 2 mm) at a flow rate of 200 μl/min. The elution solvent was 88% water/12% acetonitrile containing an overall concentration of 9 g/l sodium dihydrogen phosphate, 200 mg/l EDTA and 320 mg/l octane sulphonic acid. The pH was adjusted to 4.20 with orthophosphoric acid. Mobile phase was precleaned by passing through a guard cell, controlled via a Coulochem 5100 controller set at +450 mV, and situated between the pump and autosampler. Detection was achieved with an Antec electrochemical detector with a cell potential of +750 mV. Data were collected on a Waters Millennium^{32} chromatography data system. Dopamine, DOPAC and HVA concentrations in the samples were calculated by comparison with calibration curves constructed from pure reference standards.

2.5. Histological analysis

After behavioural testing, the animals were given an overdose of anaesthetic, the thorax opened and perfused with 30 ml of saline followed by 30 ml of 10% buffered formalin via the left ventricle or vena cava. The brains were removed, cut into 2 × 6 mm segments using a rodent brain matrix, processed and embedded in paraffin wax. Coronal sections (8 μm) were taken on a sledge microtome.

2.5.1. Tyrosine hydroxylase and growth-associated protein 43 immunocytochemistry

Briefly, the sections were deparaffinised and rehydrated and endogenous peroxidase was quenched with 0.3% H_2O_2 for 30 min. The slides were placed in pepsin (0.2 g Sigma-p-7000 pepsin in 50 ml 0.01 M HCl) for 30 min, washed and nonspecific binding was blocked with 1.5% normal goat serum (Vectastain rabbit IgG ABC kit) diluted in phosphate-buffered saline (PBS) incubated for 20 min. This was followed by application of rabbit polyclonal anti-tyrosine hydroxylase (TH) antibody (Chemicon AB152, 1:100 dilution) incubated for 18 h at room temperature. After washing in PBS (3 × 5 min), the sections were incubated with the biotinylated secondary antibody (Vectastain rabbit IgG ABC kit) for 30 min followed by 3 × 5 min washes with PBS. The horseradish peroxidase conjugate (Vectastain rabbit IgG ABC kit) was applied for 30 min followed by 3 × 5 min PBS rinses. Antibody binding was visualised using the chromagen 3, 3′-diaminobenzidine (DAB substrate kit, Vector SK-4100). The sections were dehydrated, cleared and then coverslipped using DPX mountant. Adjacent sections from the efficacy studies were stained in a similar way using a primary rabbit polyclonal antibody to growth-associated protein 43 (GAP-43) (Chemicon AB5220, 1:500 dilution).

2.5.2. Image analysis

After tyrosine hydroxylase immunostaining, the striatal slides were digitised and using an image analysis system (Optimas 5.2) the areas of the dorsal and ventral striatum of each hemisphere were outlined individually and the mean grey densities were measured. The staining intensity of each lesioned hemisphere was expressed as a percentage of the respective intact hemisphere from that animal. Sections of the substantia nigra taken at −5.00 mm caudal to bregma in rat (Paxinos and Watson, 1986) and 3.08 mm caudal to bregma in mouse (Paxinos and Franklin, 1997) were also stained for TH immunoreactivity. The number of tyrosine hydroxylase positive cells per slide in left and right substania nigra were counted at ×25 magnification. The same image analysis system was used to quantify the growth-associated protein 43 immunoreactivity. The mean grey intensity of the intact and lesioned striatum was calculated

and data was then expressed as a percentage change in growth-associated protein 43 immunoreactivity between the intact and lesioned hemisphere.

2.6. Statistics

Statistical analysis of data was carried out using analysis of variance (ANOVA) followed by appropriate post hoc test using $P < 0.05$ as the level of significance. All analysis was performed using the statistical analysis package JMP® (SAS Institute, USA).

3. Results

3.1. Neuroprotective effects of LY404187 in rodent models of Parkinson's disease

3.1.1. Prevention of MPTP-induced neurotoxicity in mice

LY404187 was administered for 11 days at 0.5 mg/kg s.c. On day 8, the mice were challenged with MPTP (20 mg/kg × 4 injections at 2 h intervals). We have previously shown that this dose of MPTP produces a rapid (within 24 h) depletion of dopamine "positive" cell bodies and terminals (O'Neill et al., unpublished data). Results indicated that treatment with LY404187 attenuated the loss of tyrosine hydroxylase immunoreactivity in the substantia nigra (Fig. 1B), but there was no significant change in tyrosine hydroxylase immunoreactivity in the dorsal and ventral striatum (Fig. 1A).

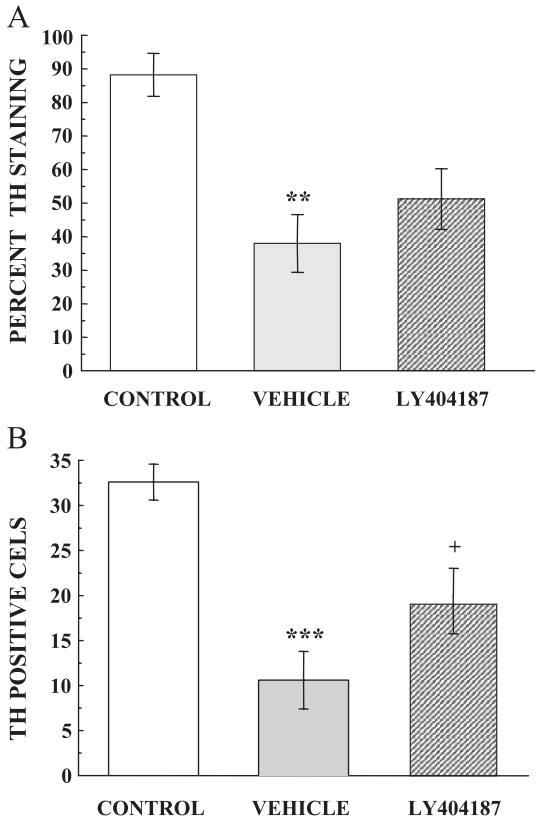

Fig. 1. Effects of pretreatment for 7 days with LY404187 (0.5 mg/kg s.c.) on the density of tyrosine hydroxylase immunoreactivity in the striatum (A) and the number of tyrosine hydroxylase positive cells in the substantia nigra (B) after MPTP treatment in mice. LY404187 provided significant protection against MPTP-induced neurotoxicity in the substantia nigra. Data are based on $n = 5–6$ per group. $^{**}P < 0.01$ vs. control, $^{+}P < 0.05$ vs. vehicle.

3.1.2. Functional and histological protection after infusion of 6-hydroxydopamine into the striatum in rats

In earlier studies we found that 10 μg of 6-hydroxydopamine infused into the striatum produces a slow, partial retrograde degeneration of the cell bodies in the substantia nigra. We observed a 50% loss in tyrosine hydroxylase positive cells at 4 weeks after infusion of 6-hydroxydopamine. In the present studies we found that 28 days treatment with LY404187 (0.5 mg/kg s.c.) attenuated apomorphine-induced contraversive rotations (Fig. 2A) and afforded significant protection against the loss of tyrosine hydroxylase positive nigral cell bodies (Fig. 2B and C). It should be pointed out that the data in Fig. 2B and C are from sections taken at one stereotaxic level and further stereological counting would be required to conclusively demonstrate protection. However, we have found similar results with another AMPA potentiator, LY503430 (Murray et al., 2003), and with both compounds the protection was associated with an improvement in function as measured using the rotometers.

3.1.3. Functional and histological protection after infusion of 6-hydroxydopamine into the substantia nigra in rats

Our preliminary work indicated that 4 μg of 6-hydroxydopamine infused into the substantia nigra produces progressive loss of cell bodies (Fig. 3A) and striatal terminals (Fig. 3B) from day 1 to day 8. The cell bodies began to die at day 1 and this is followed by the disappearance of terminals, which started at day 2. In total, the lesion produced an 85–90% loss in nigra cell bodies, 80–90% loss in of terminals in the dorsal striatum and 50–60% loss in terminals in the ventral striatum (Fig. 3B). In subsequent drug studies, we used 10–14 days to assess the extent of lesion. We carried out a series of experiments to evaluate the effects of LY404187 (0.1 or 0.5 mg/kg s.c. for 14 days, starting 1 day after 6-hydroxydopamine) on functional outcome at 16 days and histological outcome at 17 days after 6-hydroxydopamine. Results of this first experiment indicated that LY404187 provided a dose-dependent correction of apomorphine-induced rotations (Fig. 4A) and this was accompanied by a robust neuroprotection in the dorsal and ventral striatum (Fig. 4B). To illustrate the magnitude of the protection, examples of striatal sections stained for tyrosine hydroxylase from sham, lesioned and LY404187 0.5 mg/kg treated animals are shown (Fig. 5A). These effects were accompanied by only a modest effect on the number of tyrosine hydroxylase positive cells in the substantia nigra (Fig. 4C).

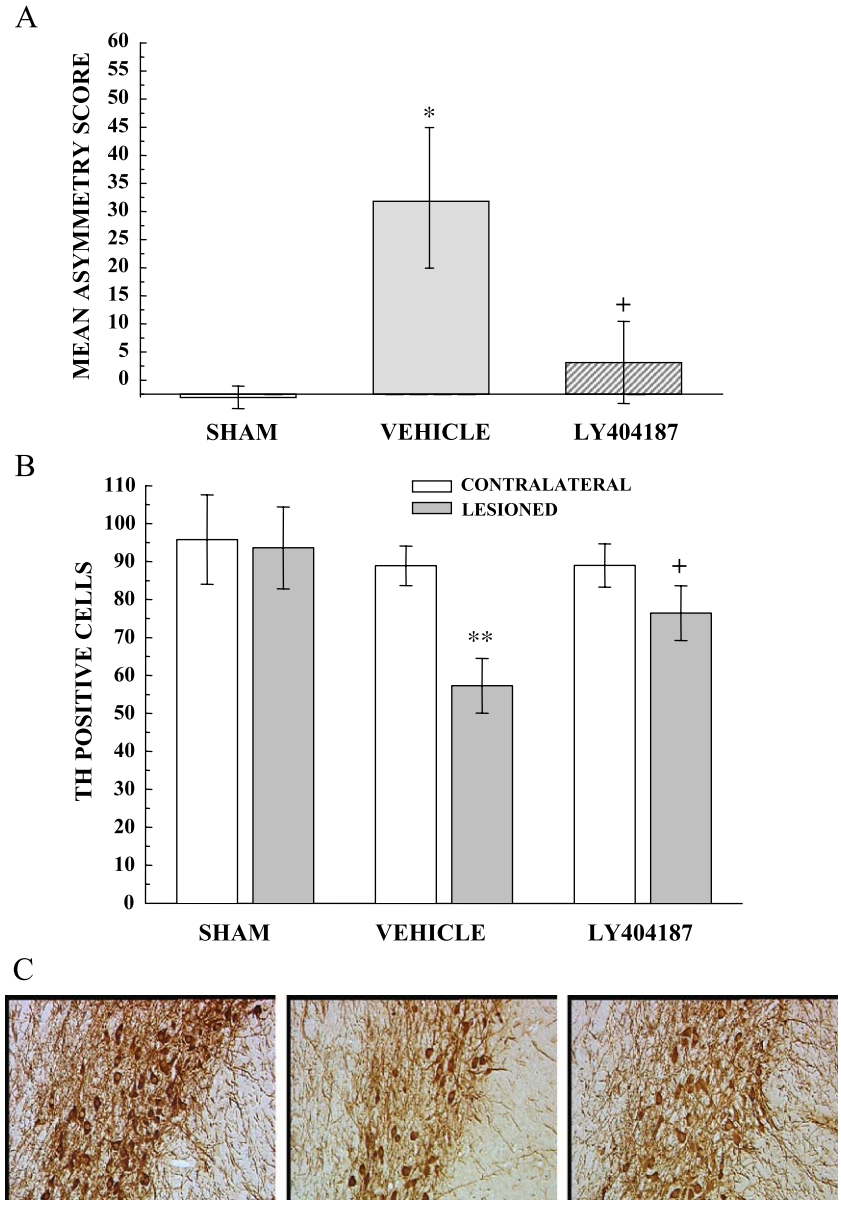

Fig. 2. The effects of chronic treatment with LY404187 (0.5 mg/kg s.c. for 28 days starting 24 h after infusion of 6-hydroxydopamine into the striatum) on (A) rotational behaviour and (B, C) tyrosine hydroxylase immunoreactive cells in the substantia nigra. Results indicate that LY404187 provided significant correction of apomorphine-induced rotational asymmetry and significant protection against loss of tyrosine hydroxylase staining seen after unilateral infusion of 6-hydroxydopamine into the striatum. Data are based on eight animals per group. *$P<0.05$, **$P<0.01$ vs. sham, $^{+}P<0.05$ vs. vehicle.

There appeared to be no effect at 0.1 mg/kg and a small, but significant ($P<0.05$) effect at 0.5 mg/kg. Additional experiments (using the same protocol) indicated that 0.5 mg/kg of LY404187 attenuated apomorphine-induced rotations (data not shown) and attenuated the lesion-induced changes in dopamine levels (Fig. 5B). When dopamine turnover was calculated by expressing (DOPAC + HVA)/DA, LY404187 almost completely blocked the lesion-induced changes in dopamine turnover. However, it should be noted that the lesion produces profound decreases in dopamine and metabolite levels so it is not clear if dopamine turnover per se is the best biochemical measure in this case. In the current studies, this level of biochemical improvement did provide very marked correction of rotational behaviour in the same animals.

Thus, we have demonstrated that LY404187 afforded functional, neurochemical and histological protection after infusion of 6-hydroxydopamine into the substantia nigra.

3.2. Neurotrophic effects of LY404187 in rodent models of Parkinson's disease

3.2.1. Effects of delayed treatment with LY404187 after unilateral nigral lesion

The lack of effect in the nigra, but positive functional and histological effects in the striatum with 0.1 mg/kg of

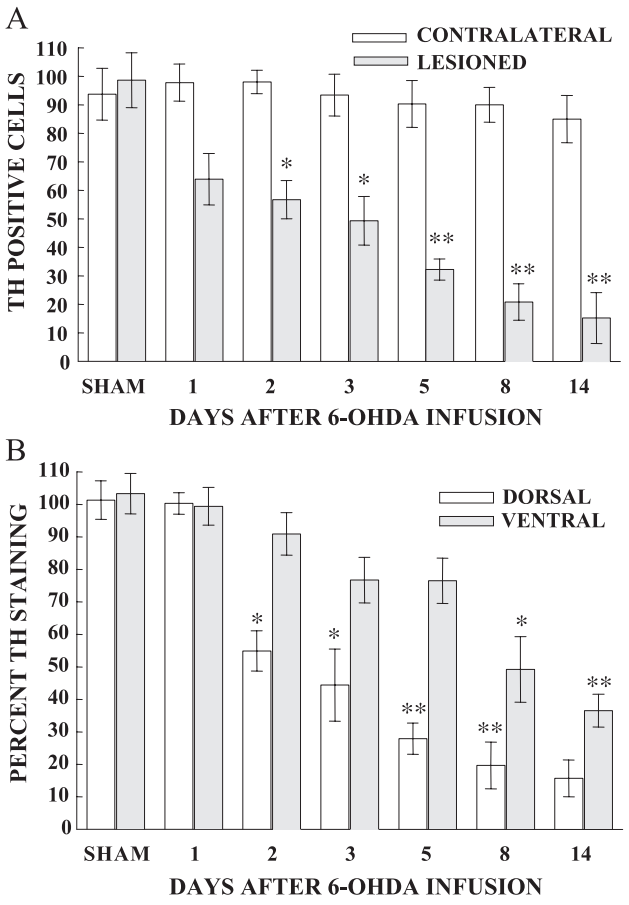

Fig. 3. Quantitative data illustrating the time course of the loss in (A) the number of nigra cells bodies and (B) the density of dopaminergic terminal tyrosine hydroxylase immunoreactivity after infusion of 4 μg of 6-hydroxydopamine into the substantia nigra. Results indicated that there is a progressive loss of cell bodies from day 1 to day 8 and this is accompanied by a slightly delayed loss in striatal terminals. $^{*}P<0.05$, $^{**}P<0.01$ vs. sham.

LY404187 suggested that the compound may have a trophic action. To investigate this further, we carried out a series of experiments where we delayed the start of treatment (LY404187 0.5 mg/kg s.c. for 14 days) until 3 or 6 days after infusion of 6-hydroxydopamine into the substantia nigra. The results indicated that delayed treatment provided an attenuation of apomorphine-induced rotations. This was significant at the 3-day time-point ($P<0.05$) and while there was a large correction at day 6, this did not reach significance due to large SEM (Fig. 6A). The results of tyrosine hydroxylase immunoreactivity from the same animals indicated that LY404187 provided a robust enhancement of terminals at both 3 and 6 days (Fig. 6B). The data strongly support a trophic action as the degree of tyrosine hydroxylase immunoreactivity in the striatum when treatment was started at 6 days (after cell death has occurred) is similar to that obtained when treatment was started at 1 or 3 days post 6-hydroxydopamine infusion. In addition, preliminary data indicated that more than 4 days of dosing is required for efficacy, even when started 1 day after lesion. This suggests that any effect we see is due to actions of down stream signalling pathways and that initiation of treatment at 6 days may mean that these pathways are not up-regulated without chronic (10–14 days) treatment with LY404187. The effect was also not due to up-regulation of tyrosine hydroxylase per se as there was no effect in control animals treated with LY404187.

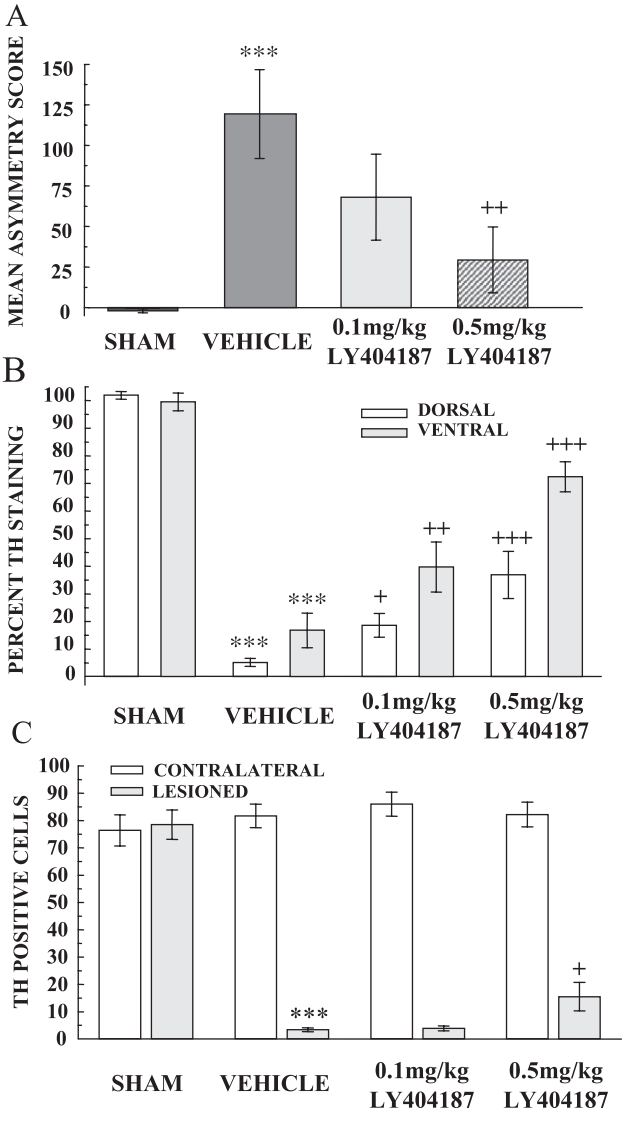

Fig. 4. The effects of chronic treatment with LY404187 (0.1 and 0.5 mg/kg s.c. for 14 days starting 1 day after infusion of 6-hydroxydopamine into the nigra) on (A) rotational behaviour, (B) tyrosine hydroxylase immunoreactivity in the dorsal and ventral striatum and (C) the number of tyrosine hydroxylase positive nigral cell bodies. Results indicate that LY404187 provided a dose-dependent correction of apomorphine-induced rotational asymmetry (A) and loss of tyrosine hydroxylase staining observed in both dorsal and ventral striatum (B) after unilateral infusion of 6-hydroxydopamine into the substantia nigra. In this study, the compound had minimal effects on the number of tyrosine hydroxylase positive cell bodies in the substantia nigra, although the higher dose did provide a significant protective effect. Data are based on eight animals per group. $^{***}P<0.001$ vs. sham, $^{+}P<0.05$, $^{++}P<0.01$, $^{+++}P<0.001$ vs. vehicle-treated animals.

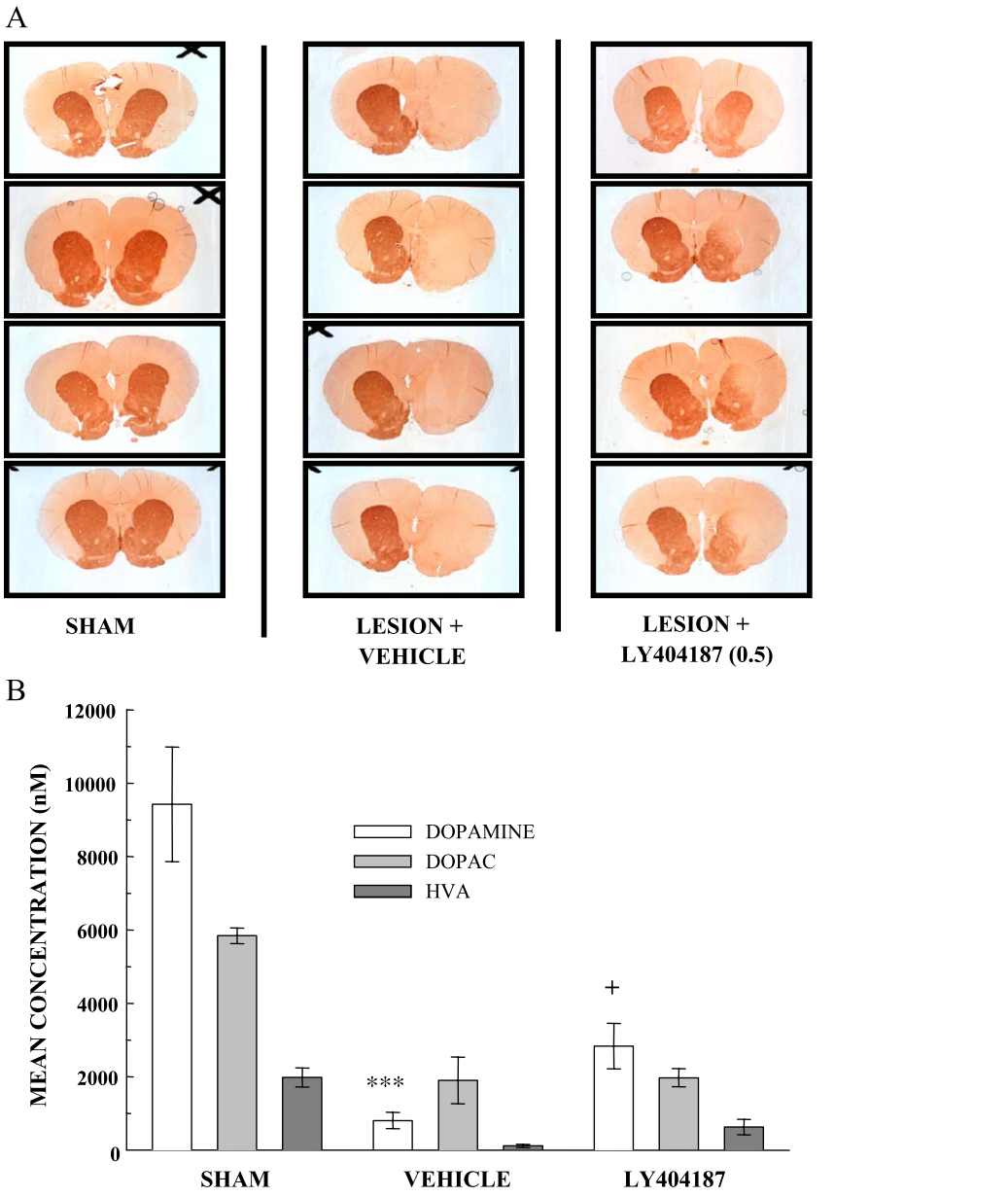

Fig. 5. Comparison of tyrosine hydroxylase immunoreactivity in the lesioned and contralateral striatum (A) and the levels of dopamine and metabolites in the lesioned striatum (B) from sham, vehicle and LY404187 (0.5 mg/kg s.c. for 14 days started 1 day after 6-hydroxydopamine). There is a clear loss in tyrosine hydroxylase immunoreactivity and an increase in dopamine turnover in striatum after 6-hydroxydopamine infusion into the substantia nigra. LY404187 provides significant attenuation of this 6-hydroxydopamine-induced loss in tyrosine hydroxylase immunoreactivity and dopamine depletion. Data are based on eight animals per group. $^{*}P<0.05$ vs. sham control, $^{+}P<0.05$ vs. vehicle control.

3.2.2. Effects of LY404187 on BDNF and GAP-43 immunoreactivity in the striatum

With some evidence that treatment with LY404187 was having a trophic action after infusion of 6-hydroxydopamine into the substantia nigra, we immunostained adjacent striatal sections for BDNF and GAP-43. We had previously shown that LY404187 (1 mg/kg for 7 days) increased BDNF protein expression in the hippocampus (Mackowiak et al., 2000). In the present studies, there was no significant difference in BDNF protein in the striatum of vehicle or drug-treated animals. This may be because the increase in BDNF is an earlier event (i.e. 4–7 days) that in turn activates pathways to enhance sprouting, the techniques were not sensitive enough to detect an increase in the striatum or that the brains were harvested 48 h after the final dose in these studies and the effect has disappeared. There was clear GAP-43 staining in the intact striatum. The 6-hydroxydopamine lesion ablated this staining in the terminal regions, and our results indicated that LY404187 provided a partial reversal of the loss in GAP-43. The

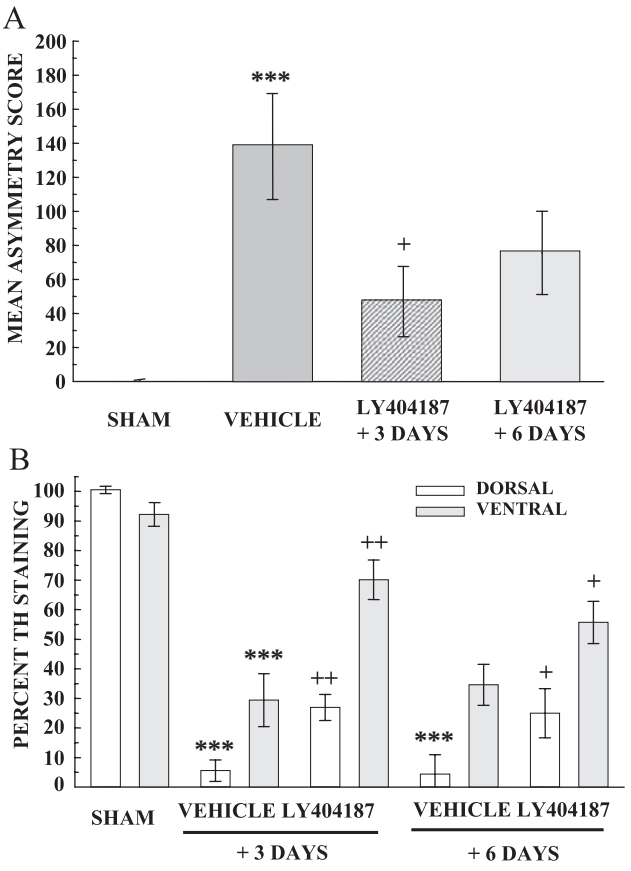

Fig. 6. Effects of delayed treatment with LY404187 (0.5 mg/kg s.c. for 14 days started either 3 or 6 days after 6-hydroxydopamine infusion) on rotational behaviour (A) and tyrosine hydroxylase immunoreactivity in the striatum (B) after a unilateral nigral 6-hydroxydopamine lesion in the rat. Results indicated that delayed treatment with LY404187 provided functional and histological improvement, suggesting a trophic action as administration was initiated after cell death. Data are based on eight animals per group. ***$P<0.01$ vs. sham control, $^{+}P<0.05$, $^{++}P<0.01$ vs. vehicle control.

increase in GAP-43 was evident only in the lesioned side of the striatum (Fig. 7). These results suggest that LY404187 is neurotrophic in this model.

4. Discussion

In the present study, we have demonstrated that a novel, potent, selective and systemically active AMPA receptor potentiator provided neurotrophic actions in three rodent models of Parkinson's disease. The compound also increased GAP-43 expression in the lesioned striatum and provided effective functional and histological protection of striatal terminals even when administration was initiated after the lesion was established.

4.1. Potency and selectivity of LY404187

Recent studies have demonstrated that the biarylopropylsulfonamide, LY404187, potentiates glutamate-induced calcium flux in human embryonic kidney 293 (HEK293) cells transfected with human iGluR receptor subtypes (Miu et al., 2001). The results indicate that LY404187 is more selective for flip vs. flop AMPA receptors ($hGlu_{A2}$ flip>$hGlu_{A4}$ flip>$hGlu_{A3}$ flip>$hGlu_{A1}$ flip>$hGlu_{A2}$ flop>$hGlu_{A1}$ flop = $hGlu_{A4}$ flop). LY404187 had EC_{50} values of 50–600 nM on the flip receptor subtypes, while in contrast CX-516 (a difference chemical class of AMPA potentiator) did not significantly increase calcium influx at concentrations up to 10 μM (Miu et al., 2001). LY404187 also potentiated AMPA currents in isolated hippocampal and purkinje neurons (Gates et al., 2001). In contrast, functional NMDA, Glu_{K5}, and Glu_{K6}, $Glu_{K6/K2}$ receptor activities in hippocampal, cortical, dorsal root ganglion and transfected HEK 293 cells as previously described (Baumbarger et al., 2001; Gates et al., 2001; Miu et al., 2001) were not affected by concentrations of 10–100 μM LY404187 and binding assays confirmed that there was no activity at 18 other neurotransmitter receptors. A detailed review of the actions of LY404187 on recombinant and native AMPA receptors was recently published (Quirk and Nisenbaun, 2002). In addition to potentiation of glutamate evoked currents in vitro, it has been shown that AMPA receptor potentiators that are related to LY404187 can increase BDNF expression in hippocampal neurons in vitro (Legutko et al., 2001). Early studies indicated that non-NMDA could regulate BDNF in the rat hippocampus (Zafra et al., 1990). More recently, it has been demonstrated that AMPA receptors interact and signal through a tyrosine kinase Lyn (Hayashi et al., 1999). It is interesting that BDNF also appears to regulate the expression of AMPA receptors in neocortical neurons (Narisawa-Saito et al., 1999a,b).

4.2. Neuroprotective actions of LY404187

It has previously been reported that systemic administration of biarylopropylsulfonamides can enhance AMPA evoked responses in hippocampal and spinal cord neurons in vivo (Vandergriff et al., 2001). These studies also demonstrated that systemic administration of LY404187 (0.01–100 μg/kg, i.v.) enhanced responses to AMPA in a dose-dependent manner. We were therefore confident that we were evaluating a potent and systemically active AMPA receptor potentiator in our Parkinson's disease studies. In the present studies, we have used well-established models with MPTP and 6-hydroxydopamine that produce cell loss and permanent dopamine depletion in vivo (Bezard et al., 1998; Gerlach and Riederer, 1999; O'Neill and Siemers, 2002). Our results indicated that LY404187 could reduce neurotoxicity whether we used systemic MPTP in mice, infusion of 6-hydroxydopamine onto the cell bodies or into the striatum or whether we used functional, neurochemical or histological end-points. Another recent study has demonstrated that AMPA receptor potentiators are neuroprotective against lesions induced by the NMDA agonist in the neonatal mouse brain (Dicou et al., 2003). The authors

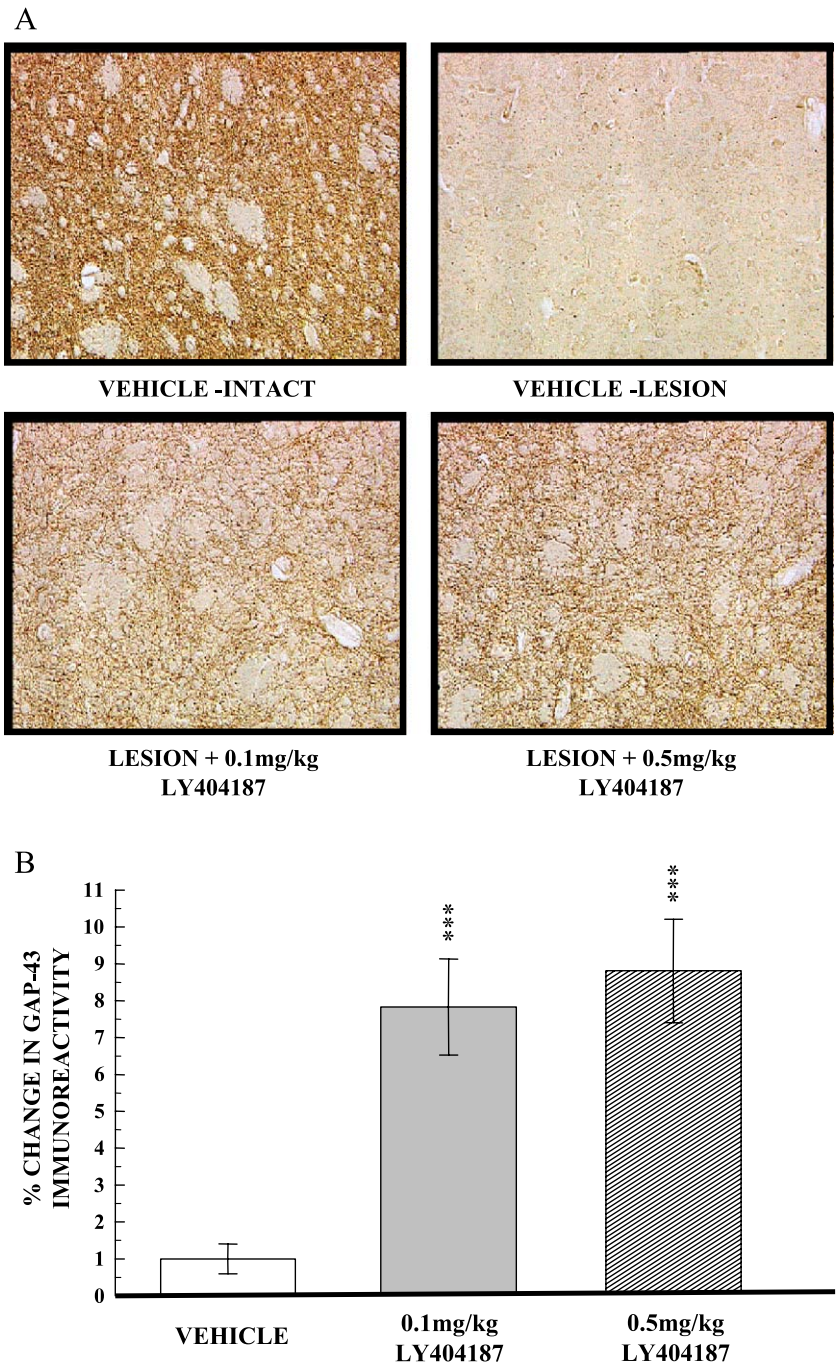

Fig. 7. Illustrates representative photomicrographs of GAP-43 immunostaining (A) from the intact striatum, lesioned striatum, lesioned + LY404187 (0.1 mg/kg) and lesioned + LY404187 (0.5 mg/kg). Dose-dependent increase in GAP-43 immunoreactivity in the striatum with LY404187 (0.1 and 0.5 mg/kg s.c. for 14 days) starting 1 day after unilateral nigral 6-hydroxydopamine lesion in the rat (B). $^{**}P < 0.01$ vs. vehicle control.

demonstrated that cyclothiazide, CX614, S18986-1 and LY404187 exerted protective against an ibotenic acid-induced lesion in the cortex. The protection was mediated via the MAPK pathway because the MEK inhibitor, PD98059, blocked the protective effects of the AMPA potentiators.

Many investigators have reported that antioxidants, nitric oxide synthase inhibitors, anti-inflammatory agents, nicotine, immunophilins and related molecules can provide protection in rodent models of Parkinson's disease. We have evaluated many of the above small molecule interventions in 6-hydroxydopamine models and our results indicate that LY404187 provides superior protection to many of these other pharmacological agents. For example, our data indicates that the dopamine receptor agonist, pramipexole, and the adenosine $A2_A$ receptor antagonist, KF17837, fail to provide any protection under the same conditions, while the $mGluR_{2/3}$ receptor agonist, LY379268 (Murray et al., 2002) and nicotine (O'Neill et al., 2002) provide some protection when

administered prior to the 6-hydroxydopamine, but no protection when delayed until 3–6 days after 6-hydroxydopamine. We have not evaluated growth factors per se (central application may make clinical trials more difficult), but it is clear from the literature that GDNF is very effective in these (6-hydroxydopamine and MPTP) models (Rosenbald et al., 2000). Furthermore, recent studies suggest functional (Unified Parkinson's Disease Rating Scale, UPDRS) and biochemical (^{18}F dopamine positron emission tomography scans) effects in Parkinson's disease patients after direct infusion of GDNF into the caudate (Gill et al., 2003).

4.3. Neurotrophic actions of LY404187

In addition to the neuroprotective actions of LY404187, we are confident that the molecule is also neurotrophic in our unilateral nigral lesion model. This is supported by the increase in GAP-43 in the lesioned animals treated with LY404187. It is well established that GAP-43 is involved in neuronal repair and sprouting (Benowitz and Routtenberg, 1997; Namgung and Routtenberg, 2000). Transgenic animals that overexpress GAP-43 exhibit increased sprouting and repair after exposure to lesions. GAP-43 is up-regulated in areas adjacent to lesions, for example, in the penumbra of a stroke during recovery. In the current studies, we observed a similar phenomenon, with an increase in GAP-43 in the lesioned hemisphere after LY404187. In addition, we found similar neurotrophic actions in the striatum whether we initiated administration 1, 3 or 6 days after infusion of 6-hydroxydopamine into the substantia nigra. Therefore, the degree of tyrosine hydroxylase immunoreactivity (preservation of terminals) is similar whether the drug is administered as cells start to die (1 day), as nigra cells are dying (1 and 3 days after 6-hydroxydopamine) or with a 80% loss of nigral cell bodies (6 days after 6-hydroxydopamine). We have previously found a similar effect with a related AMPA receptor potentiator, LY503430, in this model (Murray et al., 2003). Thus, we hypothesize that LY404187 (and LY503430) have trophic actions on the remaining nigral cell bodies and/or striatal terminals to enhance sprouting of these terminals (see Dunnett and Björkland, 1999 for review of restorative treatments in Parkinson's disease).

4.4. Mode of action of LY404187

The exact mechanism of the neuroprotective/neurotrophic effects is not clear, but an obvious mechanism may be an up-regulation of trophic factors, in particular BDNF. It is well established that AMPA receptor activation can increase BDNF gene expression (Zafra et al., 1990). These increases may be activity-dependent as it has been reported that membrane depolarization (for example, produced by activation of the AMPA receptor) facilitates the opening of voltage sensitive calcium channels and the resultant increase in intracellular calcium then increases gene expression through calcium response elements in the BDNF promoter region (Shieh and Ghosh, 1999; Tao et al., 1998). We have recently shown that LY404187 produces discrete increases in glucose utilization in the rat brain (Fowler et al., 2002) and this activation occurs at doses (0.5 mg/kg) that provide robust effects in the current studies. Another mechanism was suggested by Hayashi et al. (1999) when they reported that AMPA receptor potentiators can increase BDNF via the tyrosine kinase Lyn, which is physically associated with the AMPA receptor. It has been demonstrated that biarylpropylsulfonamides can increase BDNF expression in both cerebellar granule and hippocampal neurons in vitro (Legutko et al., 2001). The increases produced by AMPA and a combination of AMPA and an AMPA potentiator could be blocked by nimodipine, while the increases produced by an AMPA potentiator alone could be blocked by the MAP-Erk kinase inhibitor, PD 098059 (Legutko et al., 2001). These data suggest that AMPA-mediated increases in BDNF gene expression can be mediated by multiple mechanisms. Another recent study reported that the protective effects of AMPA potentiators after an ibotenic acid lesion were also prevented by PD 098059 (Dicou et al., 2003).

Furthermore, sub-chronic treatment with AMPA receptor potentiators increases BDNF mRNA (Lauterborn et al., 2000; Mackowiak et al., 2000) and protein (Mackowiak et al., 2000) in the rat hippocampus. The molecular mechanisms in calcium regulated BDNF regulation have recently been investigated (Shieh et al., 1998; Tao et al., 1998). It is now clear that BDNF can be increased by activation of CREB dependent pathways or via AMPA receptor activation. It has been shown that many small molecule antidepressants increase BDNF (Nibuya et al., 1995) and central application of BDNF (Siuciak et al., 1997) and systemic AMPA receptor potentiators (Li et al., 2001; Skolnick et al., 2001) have also been shown to have antidepressant like actions. BDNF is widely distributed in the brain (Schmidt-Kastner et al., 1996) and has been implicated in synaptic plasticity (Schinder and Poo, 2000). In addition to depression and cognition, BDNF has been implicated in brain injury (Lindvall et al., 1994) and BDNF is decreased in Parkinson's disease (Howells et al., 2000) and addition of BDNF has beneficial actions on dopaminergic cells in vitro and in vivo. We did not observe any increases in BDNF using immunocytochemical methods in nigra or striatal sections from the current efficacy studies. However, further studies are required to investigate if immunocytochemistry is of sufficient sensitivity, the time course of BDNF expression (it may occur prior to the end-points used in the current studies) and if BDNF is increased in other brain regions that project to the striatum. While we have not proven that BDNF is directly involved in our efficacy studies, as presented above there is substantial evidence linking AMPA receptor activation and BDNF and it is clear that both BDNF and GDNF can protect dopaminergic neurons in vitro and in vivo (Beck et al., 1995; Gash et al., 1996; Klein et al., 1999; Tomac et al., 1995). It is also of interest that another recent study has demonstrated that LY451646 (the

active isomer of LY404187), increased cell proliferation in the rat dentate gyrus (Bai et al., 2003).

As mentioned above, we did observe an increase in GAP-43 in both the intact and lesioned striatum of LY404187-treated animals. The magnitude of increase was larger in the lesioned striatum, suggesting that a combination of lesion and AMPA receptor potentiator is required to robustly increase GAP-43. Manipulation of GAP-43 has profound effects on neurite outgrowth in vitro and agents that increase GAP-43 accelerate functional recovery in vivo. It seems likely that LY404187 signalling through the AMPA receptor can increase neuronal activity (Fowler et al., 2002), trophic factors (Mackowiak et al., 2000), cell proliferation and neurogenesis (Bai et al., 2003) and these mechanisms enhance sprouting of striatal terminals (Murray et al., 2003).

4.5. Concluding remarks

In conclusion, we have provided strong evidence that an AMPA receptor potentiator can provide functional, neurochemical and histological protection in rodent models of Parkinson's disease. This class of molecule has also been reported to provide positive effects in models of depression (Li et al., 2001; Quirk and Nisenbaun, 2002) and cognition (Staubli et al., 1994; Hampson et al., 1998a,b; Quirk and Nisenbaun, 2002), two co-morbidities in patients with Parkinson's disease. In addition, these protective effects in the current studies were maintained when administration was delayed until after cell death and accompanied by an increase in GAP-43 expression in the striatum provided tantalizing evidence for neurotrophic action. These results suggest that LY404187 or a related analogue would be an ideal molecule to advance as a clinical candidate to halt or potentially reverse the degeneration observed in Parkinson's disease.

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