CNS Drug Reviews
Vol. 11, No. 1, pp. 77–96
© 2005 Neva Press, Branford, Connecticut

LY503430: Pharmacology, Pharmacokinetics, and Effects in Rodent Models of Parkinson's Disease

[1]Michael J. O'Neill, [1]Tracey K. Murray, [2]Michael P. Clay, [2]Terry Lindstrom, [2]Charles R. Yang, [2]Eric S. Nisenbaum

[1]Eli Lilly & Co. Ltd., Lilly Research Centre, Windlesham, Surrey, UK; [2]Eli Lilly & Co., Lilly Corporate Center, Indianapolis, USA

Keywords: 6-Hydroxydopamine — AMPA receptor potentiator — BDNF — LY503430 — MPTP — Neuroprotection — Parkinson's disease.

ABSTRACT

Glutamate is the major excitatory transmitter in the brain. Recent developments in the molecular biology and pharmacology of the α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)-subtype of glutamate receptors have led to the discovery of selective, potent and systemically active AMPA receptor potentiators. These molecules enhance synaptic transmission and play important roles in plasticity and cognitive processes. In the present studies we characterized a novel AMPA receptor potentiator, LY503430, on recombinant human $GLU_{A1–4}$ and native preparations *in vitro*, and then evaluated the potential neuroprotective effects of the molecule in rodent models of Parkinson's disease. Results indicated that at submicromolar concentrations LY503430 selectively enhanced glutamate-induced calcium influx into HEK293 cells transfected with human GLU_{A1}, GLU_{A2}, GLU_{A3}, or GLU_{A4} AMPA receptors. The molecule also potentiated AMPA-mediated responses in native cortical, hippocampal and substantia nigra neurones. LY503430 had good oral bioavailability in both rats and dogs. We also report here that LY503430 provided dose-dependent functional and histological protection in animal models of Parkinson's disease. The neurotoxicity following unilateral infusion of 6-hyrdoxydopamine (6-OHDA) into either the substantia nigra or the striatum of rats and that following systemic 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in mice were reduced. Interestingly, LY503430 also had neurotrophic actions on functional and histological outcomes when treatment was delayed until well after (6 or 14 days) the lesion was established. LY503430 also produced some increase in brain derived neurotrophic factor (BDNF) in the substantia nigra and a dose-dependent increase in growth asso-

Address correspondence and reprint requests to: Dr. Michael J. O'Neill, Eli Lilly & Co., Ltd., Lilly Research Centre, Erl Wood Manor, Windlesham, Surrey, GU20 6PH, UK.
Tel.: +44 (1276) 483547; Fax: +44 (1276) 483525. E-mail: Oneill_Michael_J@Lilly.com

ciated protein-43 (GAP-43) expression in the striatum. Therefore, we propose that AMPA receptor potentiators such as LY503430 offer the potential of a new disease modifying therapy for Parkinson's disease.

INTRODUCTION

In the last 30 years there has been a huge increase in our knowledge of the role of glutamate in the central nervous system. Glutamate acts at both ionotropic and metabotropic receptors, controls fast synaptic transmission in the brain and also plays a key role in plasticity (11,45). In the late 1980s and early 1990s a major focus of research was the study of NMDA and AMPA antagonists as neuroprotective agents for the treatment of acute ischemic injury (40). This research proved futile as the side effects profiles (14) of many of these agents and the poor preclinical and clinical study design led to disappointment for many major pharmaceutical companies (40). However, many of these research efforts provided novel pharmacological tools that helped us explore the role of various glutamate receptors in detail. This research indicated that blocking excitatory amino acid transmission produced cognitive deficits (36) and generated functional data that indicated hypoglutamatergic function in disorders such as schizophrenia (12,55,56). It is also clear that in addition to Alzheimer's disease (AD) there are cognitive deficits in schizophrenia and in neurological disorders such as stroke and late stage Parkinson's disease.

Based on this evidence, several companies have developed molecules that can boost glutamatergic transmission in the brain (20,32,41). One approach was to potentiate AMPA receptors and early molecules such as CX-516 emerged which were shown to enhance LTP (53) and memory formation (21,22,54) in rats and were progressed for human trials (31). Several newer molecules have been developed that are more potent positive allosteric modulators of AMPA receptors (41,47), and these are currently being developed as cognitive enhancers and may also have utility in other CNS disorders (32,41,61). Recent advances in our understanding of the molecular biology and gating of ionotropic glutamate receptors (25,34,48,58) are also helping with the design of better ligands.

At Eli Lilly & Co. Ltd. we have developed a novel series of biarylpropylsulfonamides (44) including LY392098, LY404187, LY503430, LY451395, and LY450108, which are potent potentiators of AMPA receptors *in vitro* (18,35), have functional CNS activity after systemic administration (4,5,60) and are active in rodent models of cognition (41,47) and depression (29,30,51). Based on the fact that these agents were able to enhance endogenous signaling in the brain (28,60), as well as to increase neurogenesis (3) and growth factor expression (28,33) we hypothesized that they may also improve outcome after CNS injury (41). Results indicated that LY503430 provided functional and histological improvement in rodent models of Parkinson's disease (PD). In the present review article, we summarize the *in vitro* profile of LY503430 at cloned and native AMPA receptors, describe its pharmacokinetics in rats and dogs and provide preclinical data in PD models that suggest that LY503430 may be useful in the treatment of this desease.

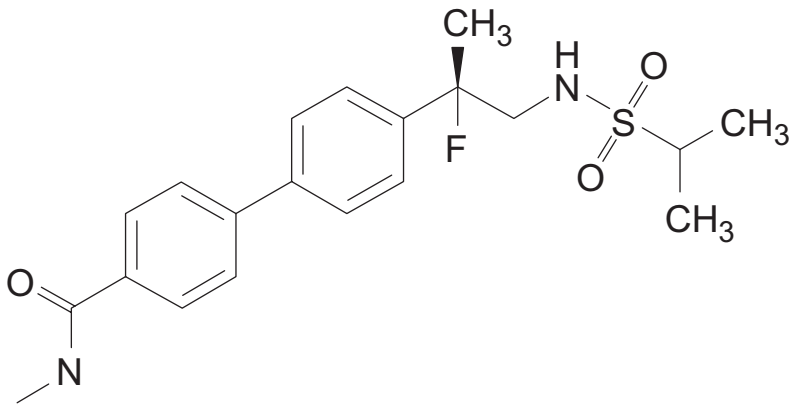

Fig. 1. The structure of LY503430.

CHEMISTRY

LY503430 (Fig. 1) is a sulfonamide with the chemical name (R)-4′-[1-fluoro-1-methyl-2-(propane-2-sulfonylamino)-ethyl]-biphenyl-4-carboxylic acid methylamide; its CAS registry number is 625820-83-9. The compound is non-hygroscopic and crystalline and is stable in solid state as well as in solutions. LY503430 has a pK_a = 6.706 ± 0.051, a log P = 1.802 ± 0.177 and its molecular weight is 392.49.

IN VITRO PHARMACOLOGY

In Vitro Effects at Cloned Human Inotropic Glutamate Receptors (iGluRs)

LY503430 has been tested on human cloned iGluR receptors expressed in HEK293 cells as described by Murray et al. (37). The compound enhanced glutamate-induced calcium influx at recombinant human $GLU_{A1–4}$ receptors. LY503430 was markedly more potent on "flip" splice variants and showed greater potency on GLU_{A2} and GLU_{A4} than on other subunits (Table 1). LY503430 had no effects on kainate-mediated responses in HEK293 cells transfected with GLU_{K5}, GLU_{K6}, or $GLU_{K6/K2}$ subunits.

In Vitro Effects on Native Tissue Preparations

LY503430 potentiated AMPA-evoked responses in a range of rat neuronal preparations (substantia nigra, cortical, Purkinje, hippocampal, and striatal neurons). When applied in the presence of AMPA, LY503430 produced concentration-dependent potentiation of the evoked current in substantia nigra (Fig. 2A) and striatal giant aspiny neurons (Fig. 2B). The EC_{50} values for LY503430 were estimated to be 2.7 μM in substantia nigra and 1.7 μM in giant aspiny neurons, respectively.

TABLE 1. In vitro *profile of LY503430 at various neurotransmitter receptors*

Receptor subtype	EC_{50} at human AMPA receptor subtypes (nM)	Crossreactivity at other glutamate receptors subtypes (μM)	Cross reactivity at other receptor subtypes (μM)
Functional effects on ion channels			
GLU_{A1} flip	475		
GLU_{A1} flop	4200		
GLU_{A2} flip	33		
GLU_{A2} flop	2250		
GLU_{A3} flip	233		
GLU_{A3} flop	3660		
GLU_{A4} flip	98		
GLU_{A4} flop	>5000		
GLU_{K5}		>100	
GLU_{K6}		>100	
$GLU_{K6/K2}$		>100	
Ca^{2+}			>10
Na^{+}			>10
K^{+}			>10
Binding to other neurotransmitter receptors			
AMPA			>100
Kainate			>100
NMDA			>100
α_1-adrenergic			>10
α_2-adrenergic			>10
β-adrenergic			>10
Dopamine D_1			>10
Dopamine D_2			>10
$5\text{-}HT_2$			>10
Histamine H_1			>10
Muscarinic			>10
Nicotinic (CNS)			>10
$GABA_A$			>10
Benzodiazepine			>10
μ-opioid			>10
κ-opioid			>10
δ-opioid			>10
Calcium L-channel			>10

Cross Reactivity with Other Neurotransmitter Receptors

LY503430 had no effect on sodium, calcium, and potassium channels measured using patch clamp electrophysiology in acutely isolated cortical neurons. In addition, LY503430 was profiled on 18 different neurotransmitter receptors using radiolabeled receptor binding assays. Assays were performed according to standard procedures available in the literature. Membrane homogenates obtained from frozen rat brain tissue or commercially

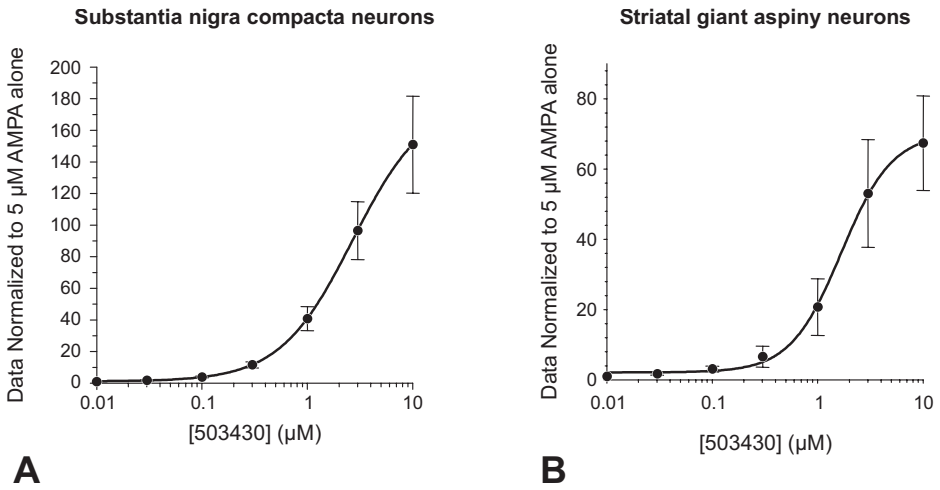

Fig. 2. Effects of LY503430 on native AMPA receptor activity *in vitro* as measured using patch clamp electrophysiology. The concentration-response profile for LY503430 (0.01–10.0 µM) potentiation was assessed by measuring the responses of acutely isolated substantia nigra dopamine neurons (**A**) or striatal giant aspiny neurons (**B**) to 5 µM AMPA alone and in the presence of a potentiator. Data points in plots represent means ± S.E.M.

available transfected cell lines were used as a receptor source. Results indicated that at 10 µM LY503430 had no affinity for the receptors listed in Table 1.

Effects on BDNF Levels in Cortical Neurons

In addition to their crucial role in synaptic transmission, activation of AMPA receptors has been reported to increase the expression of brain derived neurotrophic factor (BDNF) *in vitro* (27,28) and *in vivo* (33). This effect appears to be mediated by voltage-gated L-type calcium channels (28), activated as a consequence of the AMPA receptor-induced membrane depolarization, and by activation of Lyn, a member of the src-family of protein tyrosine kinases (23), which can physically associate with AMPA receptor subunits. LY503430 produced a large increase in BDNF levels (400 to 1000%) when applied to cortical neurons (Fig. 3), but in most experiments LY503430 had a bell-shaped dose-response curve. Similar increases in BDNF expression were also observed in hippocampal cultures treated with LY503430.

PHARMACOKINETICS

Rats

The oral bioavailability of LY503430 in rats is 84% (Table 2, Fig. 4). Male F344 rats ($n = 3$) were dosed orally with 3 mg/kg of LY503430 in a 0.5% sodium carboxymethyl cellulose/0.25% Tween 80/water vehicle, and intravenously with 0.5 mg/kg in a 5% Solutol/5% ethanol/5% propylene glycol/85% water vehicle. Plasma was collected at

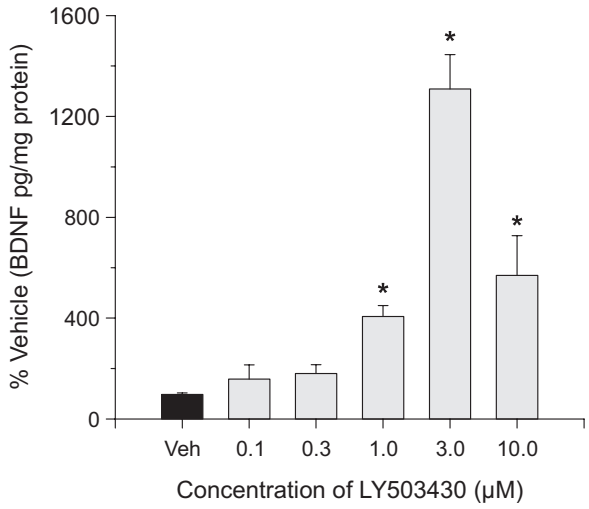

Fig. 3. The concentration-response profile for LY503430 (0.1–10.0 μM) for increase of BDNF expression in cortical neurons. * Significantly ($P < 0.05$) different from vehicle.

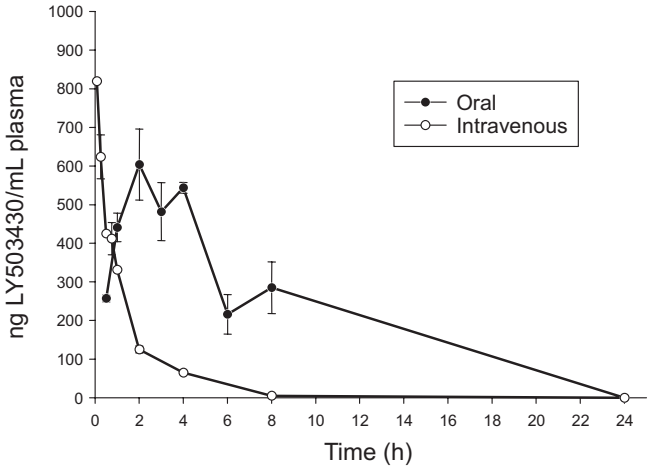

Fig. 4. Plasma levels of LY503430 in rats after a single 3 mg/kg p.o. dose or a 0.5 mg/kg i.v. dose of LY503430. Vertical bracket-like lines indicate S.E.M.

*TABLE 2. Pharmacokinetic parameters of LY503430 in rats**

Route	Dose (mg/kg)	$AUC_{0-\infty}$ (ng · h/mL)	C_{max} (ng/mL)	t_{max} (h)	$t_{1/2}$ (h)	Clearance (mL/min/kg)	F (%)
i.v.	0.5	1087	820	0.08	1.2	8	—
p.o.	3	5487	604	2	3–4	—	84

* Mean values for three animals.

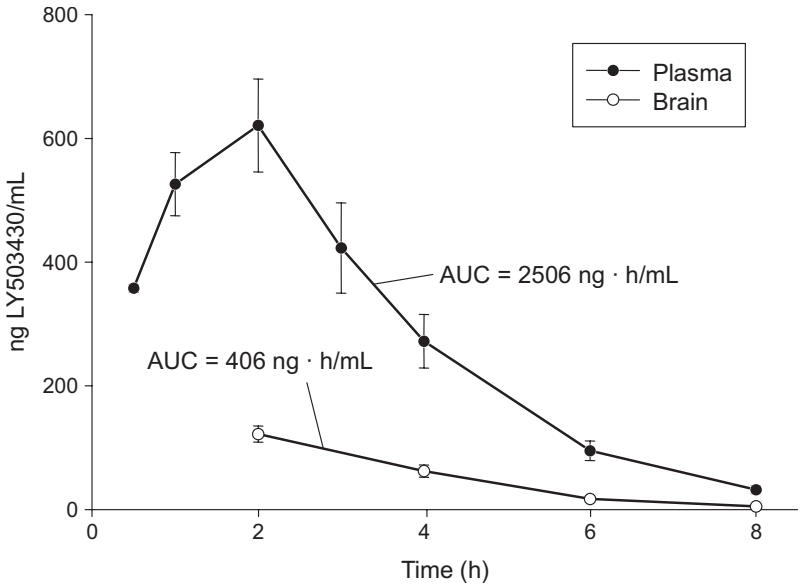

Fig. 5. Brain and plasma levels of LY503430 in rats ($n = 3$) after a single 3 mg/kg p.o. dose of LY503430. Vertical bracket-like lines indicate S.E.M.

regular intervals over 24 h after treatment. The plasma elimination half-life was 1.2 hours after intravenous and 3 to 4 h after oral treatment.

Rat brain levels of LY503430 were approximately 20% of the plasma levels over the eight hour post-dosing interval as shown in Table 3 and Fig. 5. The half-life of LY503430 in brain was ~1 h.

Dogs

The oral bioavailability of LY503430 in dogs is 66 ± 15% (Table 4, Fig. 6). Dogs (2 males and 1 female) were dosed orally with 1 mg/kg of LY503430 in a 0.5% sodium carboxymethyl cellulose/0.25% Tween 80/water vehicle, and intravenously with 0.1 mg/kg of LY503430 in a 5% Solutol/5% ethanol/5% propylene glycol/85% water vehicle. Plasma was collected over the 0.5–24 h post dosing interval. The mean plasma elimination half-life was two hours after intravenous dosing and 4.7 h after oral dosing.

*TABLE 3. Brain and plasma levels of LY503430 in rats**

	Time after treatment (h)			
	2	4	6	8
Brain (ng/g)	122 ± 13	62 ± 10	17 ± 3	5 ± 3
Plasma (ng/mL)	621 ± 75	272 ± 43	95 ± 16	32 ± 6
Brain/plasma	0.2	0.2	0.2	0.2

* Mean values (± S.E.M.) for 3 rats at hours after single 3 mg/kg oral dose of the drug.

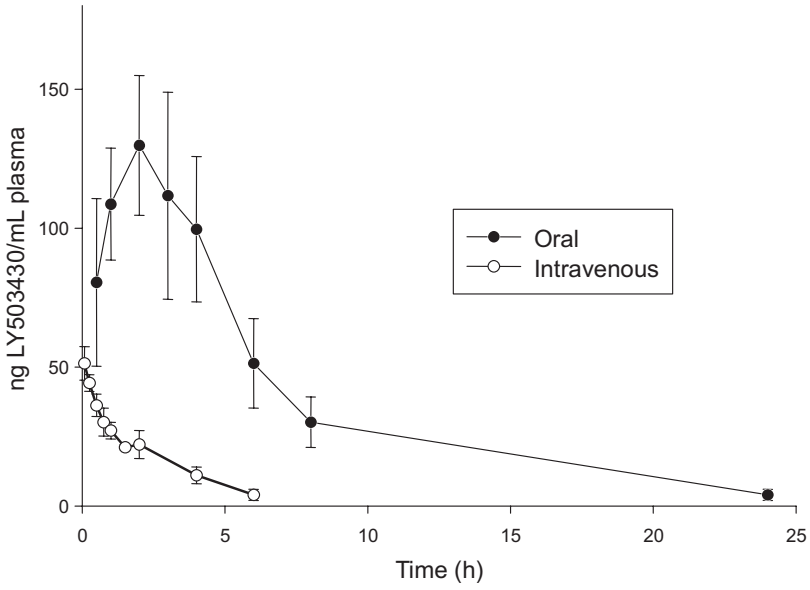

Fig. 6. Plasma levels of LY503430 in dogs after a single 1 mg/kg p.o. dose or a 0.1 mg/kg i.v. dose of LY503430. Vertical bracket-like lines indicate S.E.M.

IN VIVO PHARMACOLOGY

In Vivo Effects of LY503430 on Prefrontal Cortical Neurons

LY503430 (0.01–10 μg/kg i.v.), potentiated the probability of spike discharge evoked in prefrontal cortical (PFC) neurons by submaximal stimulation of the ventral subiculum in a dose-dependent manner. The threshold dose (0.1 μg/kg) for potentiation by LY503430 of synaptic responses in PFC (Fig. 7) was identical to that for potentiation of iontophoretically applied AMPA-evoked firing of CA1 hippocampal neurons. In contrast to the effects on synaptic responses, LY503430 did not significantly enhance the spontaneous activity of PFC neurons. Furthermore, by systemic administration LY503430 (0.01–10 μg/kg, i.v.) also enhanced responses of hippocampal neurons to iontophoretically applied AMPA in a dose-dependent manner (37). These data indicate that the brain levels of LY503430 after i.v. administration of the drug at the above listed doses were sufficiently high to produce functional effects.

*TABLE 4. Pharmacokinetic parameters of LY503430 in dogs**

Route	Dose (mg/kg)	AUC_{0-24} (ng · h/mL)	C_{max} (ng/mL)	t_{max} (h)	$t_{1/2}$ (h)	Clearance (mL/min/kg)	*F* (%)
i.v.	0.1	150 ± 8	55 ± 7	0.08	2.0 ± 0.3	11	—
p.o.	1	974 ± 177	142 ± 25	1.7 ± 0.7	4.7 ± 1.2	—	66 ± 15

* Mean values ± S.E.M. for 3 dogs after i.v. or p.o. administration of LY503430.

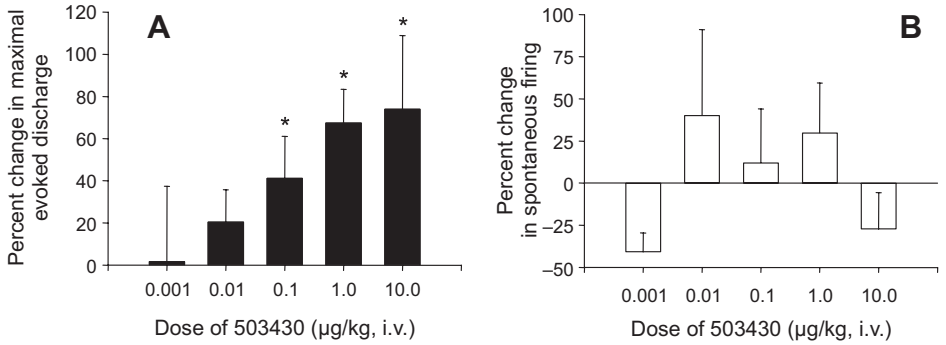

Fig. 7. Histograms summarizing the effects of LY503430 on evoked synaptic responses to submaximal hippocampal stimulation (**A**) and the spontaneous firing rate in the single PFC neurons (**B**). Note that LY503430 potentiated the hippocampal evoked PFC synaptic responses dose-dependently (with the threshold dose at 0.1 µg/kg) (**A**), but this AMPA potentiator did not change significantly the spontaneous firing rate of the same PFC neurons (**B**). * Significantly ($P < 0.05$) different from control. Vertical bracket-like lines indicate S.E.M.

Effects in Parkinson's Disease (PD) Models

LY503430 was studied in a variety of rodent models of Parkinson's disease (62). The compound had no acute effects on rotational behavior (either alone or in combination with L-DOPA or pergolide) but provided robust functional and histological protection after chronic administration. In this section some of the data generated in rodent PD models are reviewed.

Acute effects on rotational behavior

LY503430 (0.5 mg/kg s.c.) was evaluated for acute effects on baseline rotational behavior after a unilateral nigral 6-hydroxydopamine (6-OHDA) lesion in rats. In all experiments 4 µg of 6-OHDA was infused. At this dose 6-OHDA produced a large loss in dopaminergic neurons within 4 to 7 days (42). In all cases the rats were returned to home cages for 14 days before behavioral testing. Only rats that showed good responses to amphetamine or apomorphine were used in the assessment of new drugs (including LY503430). Acute treatment with LY503430 (0.5 mg/kg s.c.) had no effect on asymmetry scores (Fig. 8B). The effects have been compared with those of amphetamine in the same animals (Fig. 8B).

In order to assess the effects of LY503430 on rotational behavior during dopaminergic stimulation, the acute effects of the drug in combination with L-DOPA or pergolide were evaluated. Baseline rotations were measured and the rats then received L-DOPA or pergolide and 20 min later were given LY503430 and rotations measured for a further 110 min. The results indicated that both L-DOPA and pergolide produced a robust rotational response, and that this response was not altered by LY503430 (Fig. 9).

In a final series of studies unilateral lesioned rats were sensitized with amphetamine and the effects of LY503430 studied after 7 days of sensitization. Data indicated that at 0.5 mg/kg LY503430 did not alter amphetamine-sensitized rotational behavior (Fig. 10). Recently, Hess and co-workers (24) reported that AMPA potentiators (CX546 and CX614) from a different class were able to alter methamphetamine-induced circling behavior in methamphetamine-sensitized rats (24). This is of interest, since it suggests that two dif-

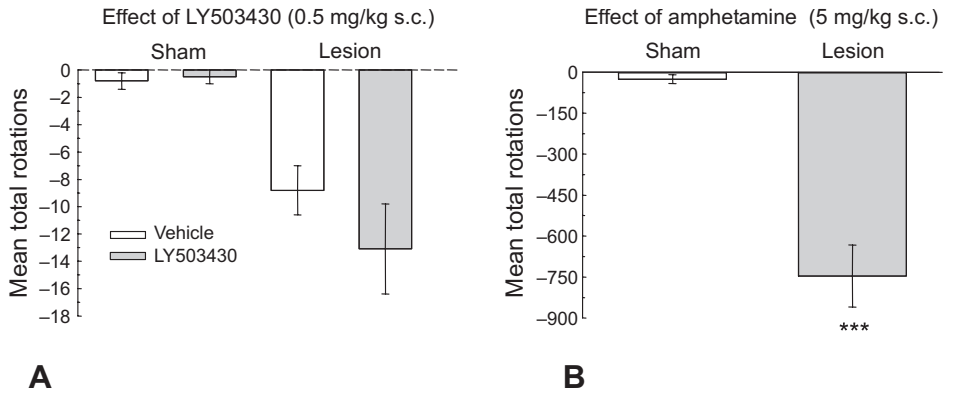

Fig. 8. The effects of acute administration of LY503430 (**A**) or amphetamine (**B**) on rotational behavior of rats one month after unilateral infusion of 6-hydroxydopamine into the substantia nigra. Amphetamine produced a robust negative asymmetry score, while LY503430 had no effect on rotational counts. ***$p < 0.001$ vs. non-lesioned. Vertical bracket-like lines indicate S.E.M.

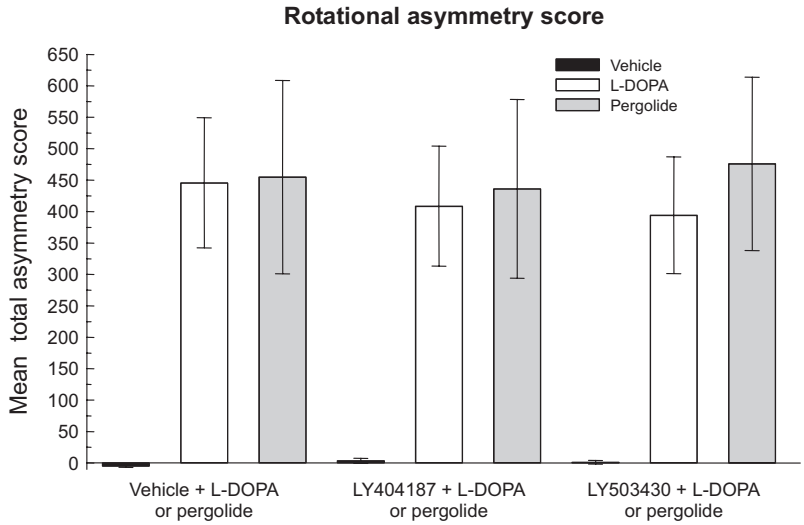

Fig. 9. The effects of LY503430 (0.5 mg/kg s.c.) and LY404187 (0.5 mg/kg s.c.) on basal and L-DOPA-(25 mg/kg i.p. ± 6.25 mg/kg benserazide i.p.) or pergolide (0.1 mg/kg i.p.)-stimulated rotations. Results indicated that acute injection of LY503430 or LY404187 had no effect on the rotational behavior induced by L-DOPA or pergolide. Vertical bracket-like lines indicate S.E.M.

ferent chemical classes of potentiators (from Cortex and Lilly) may have different functional effects in some brain areas.

Taken together, the data suggest that LY503430 has no acute effects on rotational behavior. This is not unexpected as there is little evidence that acute activation of AMPA receptors increases dopamine release. Therefore, LY503430 would not be expected to have any acute symptomatic effects in PD.

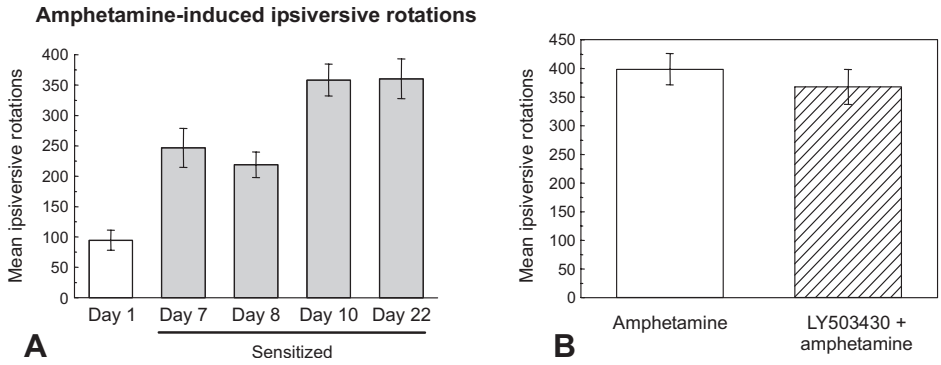

Fig. 10. The effects of LY503430 on amphetamine-sensitized rotational behavior. Rats were lesioned and only rats with apomorphine-induced rotations greater that 30 per 10 min were selected for entry into the study. Animals were sensitized for 7 days with 1.25 mg/kg amphetamine i.p. (**A**) and then tested with amphetamine alone or amphetamine ± LY503430 (0.5 mg/kg s.c.). Data indicated that LY503430 had no effect on amphetamine-sensitized rotations (**B**). Vertcal bracket-like lines indicate S.E.M.

Effects in a mouse MPTP model

In 1979, several young adults in the California region were hospitalized with motor problems of an unknown cause: resting tremor, bradykinesia, rigidity, and postural instability (13,26). These symptoms are classic hallmarks of Parkinson's disease and they responded well to L-DOPA, the primary therapy used in PD. It was subsequently discovered that these individuals had received MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) as a contaminant of the drugs they were abusing. It was then determined that MPTP is a potent neurotoxin even at low concentrations (13,26). Post-mortem studies carried out when some of those afflicted died from drug overdose revealed that damage to the brain induced by MPTP was remarkably similar to the neurodegeneration observed in PD. Since then many models of PD have been developed using MPTP administered to mice or non-human primates (6,46,52). The majority of MPTP animal models use acute administration at doses such as 4 injections of 20 mg/kg (in mice) over one day to produce damage to nigrostriatal dopaminergic neurons and achieve a model of end-phase PD within a few days (8,9). We used this model to assess the effects of LY503430 and a related compound LY404187. LY503430, daily for 11 days at 0.5 mg/kg s.c., prevented the loss of tyrosine hydroxylase immunoreactivity (TH-IR) in the striatum (Fig. 11A) and substantia nigra (Fig. 11B) of MPTP-treated mice. Since it has been reported that the severe dopamine depletion in this "acute" MPTP model is transient and animals recover over time, we also developed a "subchronic" model of MPTP in which the toxin is administered at 30 mg/kg for 8 days. Using this model we found a significant protective action of LY503430 that was administered using the same protocol. These data provide evidence that LY503430 can be neuroprotective in various mouse MPTP-induced neurotoxicity models.

Effects in a retrograde 6-OHDA model

LY503430 was evaluated for its ability to protect against damage to nigral cells following infusion of 6-OHDA into the striatum. In the initial studies we found that 10 μg of 6-OHDA infused unilaterally into the striatum produces a slow, partial retrograde degener-

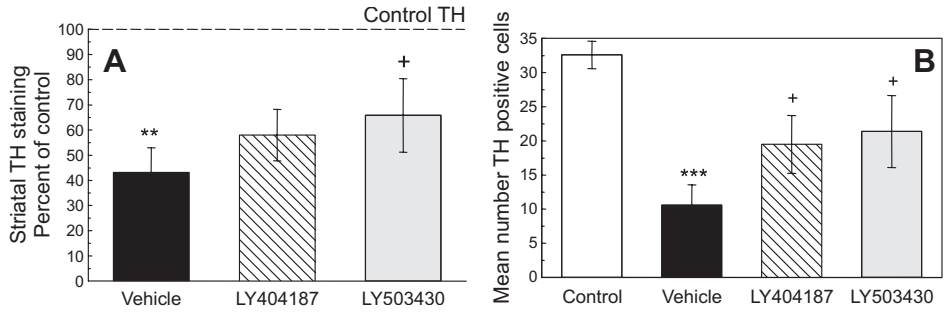

Fig. 11. Effects of pretreatment for 7 days with LY503430 or LY404187 (0.5 mg/kg s.c.) on the density of TH immunoreactivity in the striatum (**A**) and the number of TH positive intact cells per slide in the substantia nigra at –3.08 mm caudal to bregma (**B**) after MPTP treatment in mice. Both LY503430 and LY404187 provided significant protection against the MPTP-induced neurotoxicity. $n = 6$ per group. $^{**}P < 0.01$, $^{***}p < 0.001$ vs. control TH, $^{+}p < 0.05$, $^{++}p < 0.01$ vs. vehicle treated animals. Vertical bracket-like lines indicate S.E.M.

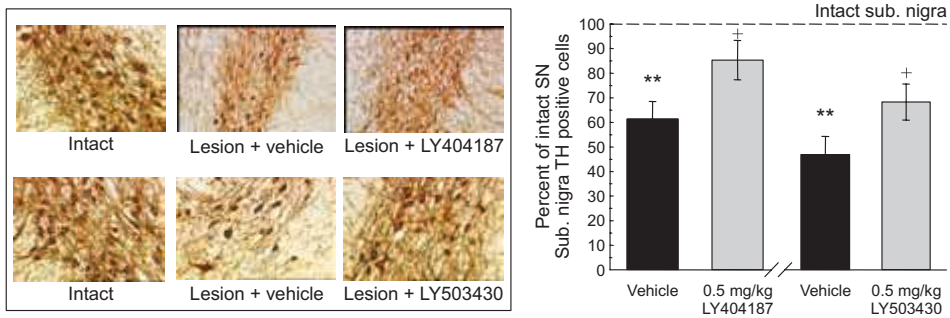

Fig. 12. The effects of LY503430 or LY404187 (0.5 mg/kg s.c. for 28 days starting 1 day after infusion of 6-OHDA into the striatum) on the number of TH immunoreactive cell bodies per slide in the substantia nigra at 5.00 mm caudal to bregma (magnification of 125×). Both LY503430 and LY404187 provided protection against nigral loss (**A**) and the number of intact TH immunoreactive nigral cell bodies was significantly higher in LY503430 and LY404187 treated animals ($p < 0.05$). Data are based on 8 animals per group. $^{**}p < 0.01$ vs. sham control, $^{+}p < 0.05$ vs. vehicle control. Vertical bracket-like lines indicated S.E.M.

ation of the cell bodies in the substantia nigra resulting in an approximate 50% loss in tyrosine hydroxylase (TH) positive cells at 4 weeks and marked ipsiversive rotations in response to amphetamine (5 mg/kg i.p.). This partial retrograde model is thought to mimic some aspects of PD as it has a slow progressive nature. Using this model, we found that dosing with LY503430 (0.5 mg/kg s.c.) for 28 days attenuated amphetamine-induced ipsiversive rotations and provided significant protection against the loss of TH positive nigral cell bodies (Fig. 12). Similar protection was observed with the related AMPA receptor potentiator LY404187 suggesting that this class of potentiators is capable of providing neuroprotection in this model.

Effects in unilateral 6-OHDA nigral lesion model

Our laboratory had also established a more severe 6-hydroxydopamine model, and LY503430 was evaluated in several studies using this model In initial studies, 4 μg of

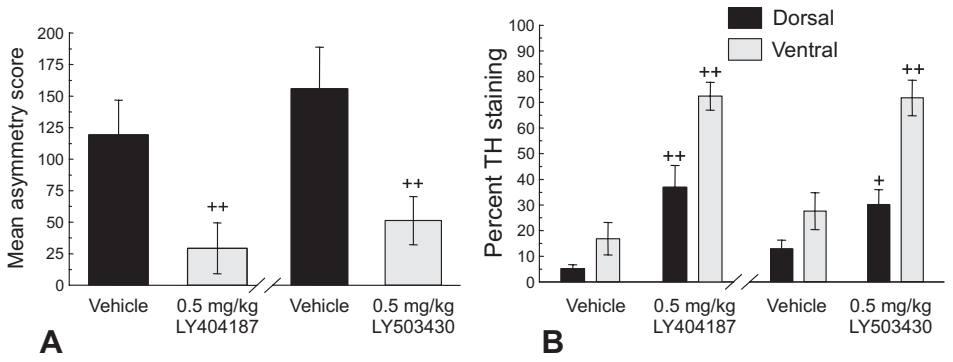

Fig. 13. The effects of chronic treatment with LY503430 or LY404187 (0.5 mg/kg s.c. for 14 days starting 1 day after infusion of 6-OHDA into the substantia nigra) on (**A**) rotational behavior and (**B**) tyrosine hydroxylase immunoreactivity in the dorsal and ventral striatum. Results indicate that both LY503430 and LY404187 provided a significant correction of apomorphine-induced rotational asymmetry (**A**) and loss of TH staining (**B**) in both dorsal and ventral striatum. Data are based on 8 animals per group. ***$p < 0.001$ vs. baseline rotations or TH, $^{+}p < 0.05$, $^{++}p < 0.01$ vs. vehicle treated animals. Vertical bracket-like lines indicate S.E.M.

6-OHDA infused into the substantia nigra produced a loss of cell bodies over the next 4 days and striatal terminals over the next 5 to 6 days, resulting in an 85–90% loss in nigra cell bodies, 80 to 90% loss of TH-IR in the dorsal striatum and 50–60% loss of TH-IR in the ventral striatum.

Subcutaneous and oral efficacy studies

Using this model, we initially demonstrated that LY503430 (or LY404187) administered at 0.5 mg/kg s.c. for 14 days starting one day after 6-OHDA was able to provide functional improvements (Fig. 13A) and associated preservation of striatal dopaminergic terminals (Fig. 13B). We then carried out a series of experiments to evaluate the effects of LY503430 (0.05, 0.1, 0.2, and 0.5 mg/kg p.o. for 10 days, starting on the first day after 6-OHDA) on functional outcome at 12 to 14 days and histological outcome at 13 to 15 days after 6-OHDA. Results of the first experiment indicated that at 0.2 or 0.5 mg/kg p.o. LY503430 prevented apomorphine-induced rotations (Fig. 14A) and provided significant protection in the dorsal and ventral striatum (Fig. 14B). Further examination of the dose-response studies indicated that the minimal effective dose was 0.08 mg/kg p.o. These effects were accompanied by only a modest effect on the number of TH positive cells in the substantia nigra (37).

Effects of delayed treatments with LY503430

The large functional improvements and preservation of striatal terminals suggested that the compound had a trophic mechanism of action. To explore this further we carried out an additional series of experiments in which the treatment with LY503430 was delayed for various time intervals after 6-OHDA. Results indicated that LY503430 was able to provide functional and histological benefits when administration began at 3 or 6 days after infusion of 6-OHDA (Fig. 15). There was also a significant effect when treatment was

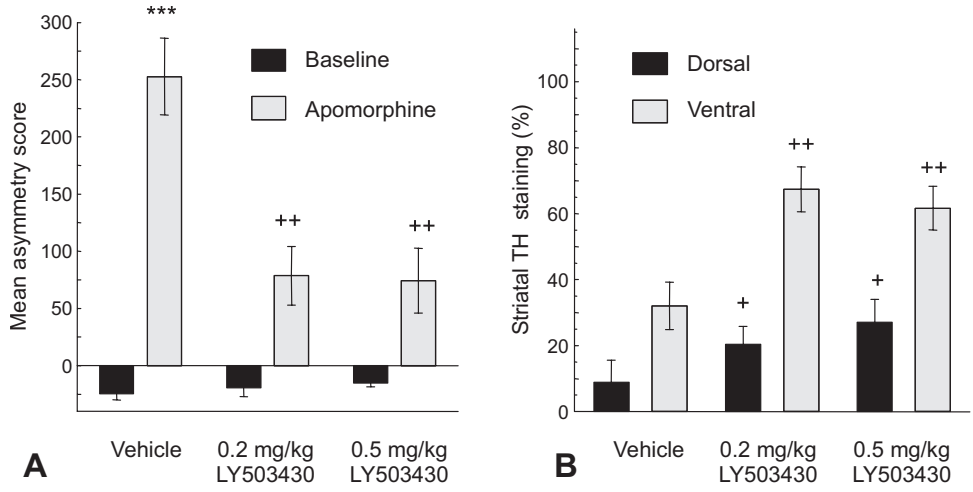

Fig. 14. The effects of chronic treatment with LY503430 (0.2 and 0.5 mg/kg p.o. for 10 days starting 1 day after infusion of 6-OHDA into the substntia nigra) on: (**A**) rotational behavior; and (**B**) tyrosine hydroxylase immunoreactivity in the dorsal and ventral striatum. Results indicate that at either dose LY503430 provided a significant correction of apomorphine-induced rotational asymmetry (**A**) and loss of TH staining in both dorsal and ventral striatum (**B**) after unilateral infusion of 6-OHDA into the substantia nigra. Data are based on 8 animals per group. $^{***}p < 0.001$ vs. baseline rotations or TH, $^{+}p < 0.05$, $^{++}p < 0.01$ vs. vehicle treated animals. Vertical bracket-like lines indicate S.E.M.

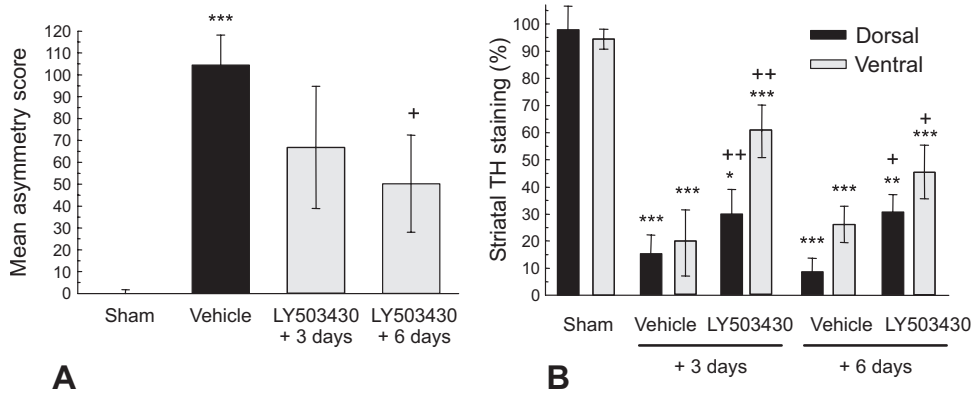

Fig. 15. The effects of delayed treatment with LY503430 (0.5 mg/kg s.c. for 14 days starting either 3 or 6 days after infusion of 6-OHDA into the substantia nigra) on: (**A**) rotational behavior; and (**B**) tyrosine hydroxylase immunoreactivity in the dorsal and ventral striatum. Results indicate that LY503430 provided a correction (significant at 6 day time point) of apomorphine-induced rotational asymmetry (**A**) and a significant protection against the loss of TH staining observed (**B**) in both dorsal and ventral striatum after unilateral infusion of 6-OHDA into the substantia nigra. Data are based on 8–10 animals per group. $^{***}p < 0.001$ vs. baseline/sham rotations or TH, $^{+}p < 0.05$, $^{++}p < 0.01$ vs. vehicle treated animals. Vertical bracket-like lines indicate S.E.M.

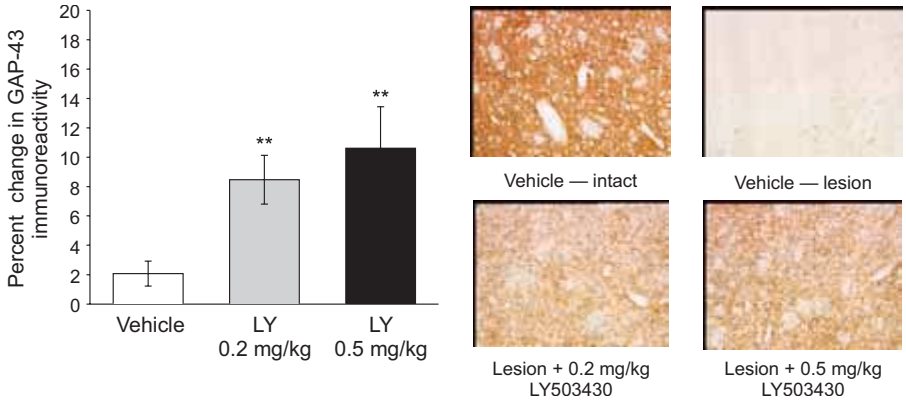

Fig. 16. Dose-dependent increase in GAP-43 immunoreactivity in the striatum with LY503430 (0.2 or 0.5 mg/kg p.o. for 10 days) starting 1 day after unilateral nigral 6-OHDA lesion in the rat. $^{**}p < 0.01$ vs. vehicle control.

started at 14 days after lesion (37). These data suggest that LY503430 is acting on either the remaining substantia nigra neurons, the striatum or other brain regions, to activate these areas or to promote reinnervation of the terminal dopaminergic areas.

Effects on growth factors and neurite outgrowth

Several adjacent sections of striatum were harvested from the oral dose-response and efficacy studies mentioned above. These sections were immunostained for a range of growth factors and growth associated protein–43 (GAP-43), a marker of neurite outgrowth (7). Results indicated that there were no significant differences in growth factor expression in the striatum between 6-OHDA lesioned animals treated with vehicle or with LY503430 at 12 days. However, there was a dose-dependent increase in GAP-43 in the lesioned striatum with LY503430 (Fig. 16). This finding suggests that new terminals have been formed and explains the large improvement in functional outcome. We also observed some increases in BNDF expression in the substantia nigra after LY503430 (37) and in the hippocampus after LY404187 (33). It is possible that to further understand the mechanisms involved more detailed evaluation of transcription and growth factors at earlier time points is required.

Washout study with LY503430

In another experiment we administered LY503430 for 14 days starting one day after 6-OHDA and then stopped treatment (washout). Animals returned to their home cages and were left untreated for the following 28 days prior to evaluating the functional and histological outcome. Using this experimental design we observed significant functional and histological improvements (Fig. 17), suggesting that chronic dosing with LY503430 increases terminal outgrowth and that this effect persists for some time. The data do not mean, however, that only a short period of treatment is required for long lasting effects in

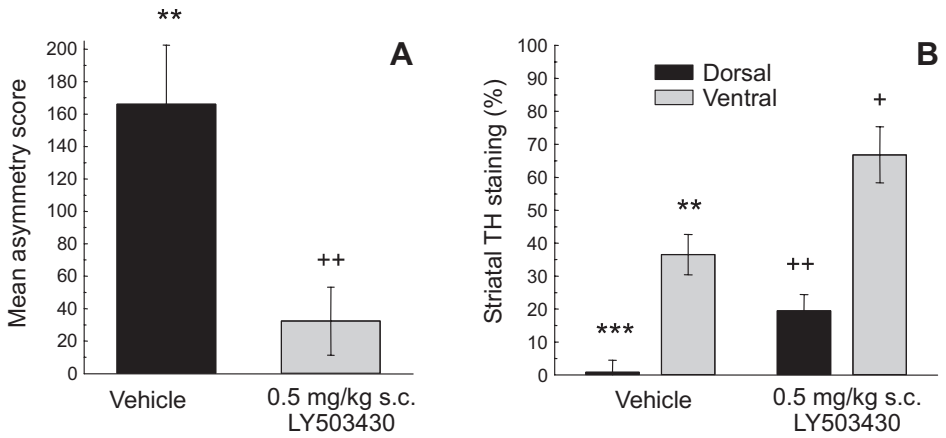

Fig. 17. The effects of chronic treatment with LY503430 (0.5 mg/kg s.c. for 14 days starting 1 day after infusion of 6-OHDA into the substantia nigra) on: (**A**) rotational behavior; and (**B**) tyrosine hydroxylase immunoreactivity in the dorsal and ventral striatum after a 28 day "washout" period. Results indicate that LY503430 provided a significant correction of apomorphine-induced rotational asymmetry (**A**) and loss of TH staining (**B**). Data are based on 8–10 animals per group. $^{**}p < 0.01$ vs. baseline rotations or TH, $^{+}p < 0.05$, $^{++}p < 0.01$ vs. vehicle treated animals. Vertical-bracket-like lines indicate S.E.M.

human PD, because in the current study the toxin was administered once and then removed from the system, while in PD there is a progressive degeneration.

DISCUSSION AND SUMMARY

Parkinson's disease (PD) is a progressive, neurodegenerative disorder of the basal ganglia. There is a large loss of dopaminergic cells in the substantia nigra, which project to the terminal rich caudate and putamen of the corpus striatum. Clinical symptoms are manifested when approximately 60% of cell bodies have degenerated and initial symptoms of PD include tremor at rest, muscular rigidity, bradykinesia, postural abnormalities and instability. The available pharmacotherapies involve dopamine replacement. These drugs reduce symptom severity, but do not dramatically affect disease progression. Therefore, in order to maintain an acceptable quality of life for patients with PD, therapies that slow or stop disease progression are needed (15,43,49).

A variety of growth factors, including glial derived neurotrophic factor (GDNF) and BDNF, have been reported to promote the survival of dopaminergic neurons in culture (38) and to protect against neurotoxin-induced lesions of the nigrostriatal system (1,2,17, 50). A major drawback of growth factors as therapeutic agents is the need for their central application. Many investigators have reported that antioxidants, nitric oxide synthase inhibitors, antiinflammatory agents, nicotine, immunophilins, and related molecules can provide protection in rodent models of PD (15,43,49).

Another approach would be to boost endogenous neurogenesis, plasticity and growth factor expression with small molecules. The data presented in this review suggest that

AMPA receptor potentiators such as LY503430 may act by this mechanism. It is possible that the reinnervation and sprouting of the dopamine-depleted striatum in the rodent model may also occur in PD patients. If so, an agent such as LY503430 while not producing any acute symptomatic effect may over time increase striatal innervation, slow disease progression and improve function. Other recent studies have reported that AMPA can protect cultured neurons against glutamate excitotoxicity through a phosphatidylinositol 3-kinase-dependent activation of extracellular signal-regulated kinase leading to upregulation of BDNF gene expression (59) and that AMPA receptor potentiators are neuroprotective against lesions in neonatal mouse brain (16).

Advances in imaging techniques have allowed clinical trials to assess disease progression (10). The REAL PET study suggested that there was slower progression of Parkinson's disease with ropinirole versus levodopa (57). The technology can also be used to visualize new dopaminergic terminals following infusion of GDNF in Parkinson's disease (19) and functional receptors after fetal dopaminergic transplantation (39). It should, therefore, be possible to study the disease modifying (neuroprotective) or enhanced sprouting (neurotrophic) actions with agents such as LY503430 in the clinical situation.

CONCLUSIONS

LY503430 is a novel brain penetrant AMPA receptor potentiator. The compound provides robust functional and histological benefits in rodent models of PD. These effects appear to be neurotrophic in nature and, therefore, agents such as LY503430 may be able to slow progression or enhance brain repair and may offer a new approach to the treatment of PD.

Addendum. Chemical names of compounds mentioned in the text:
AMPA, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid; **MPTP**, 1-methyl-4-phenyl-1,2,3, 6-tetrahydropyridine; **6-OHDA**, 6-hydroxydopamine; **GAP-43**, growth associated protein-43; **BDNF**, brain derived neurotrophic factor; **MAPK**, mitogen activated protein kinase; **GDNF**, glial derived growth factor; **CX-516**, 1-(quinoxalin-6-ylcarbon-yl)piperidine; **CX-546**, 1-(1,4-benzodioxan-6-ylcarbonyl)piperdine; **CX-614**, 2H,3H,6aH-pyrrolidino[2″,1″-3′,2′]-1,3-oxazino[6′,5′-5,4]benzo[e]1,4-dioxan-10-one; **LY404187**, N-2-(4-(4-cyanophenyl)phenyl)propyl 2-propanesulfonamide; **LY392098**, N-2-(4-(3-thienyl)phenyl)propyl 2-propanesulfonamide; **LY503430**, (R)-4′-[1-fluoro-1-methyl-2-(propane-2-sulfonylamino)-ethyl]-biphenyl-4-carboxylic acid methylamide; **LY450108**, N-[4-[(1R)-1-methyl-2-[[(1-methylethyl)sulfonyl]amino]ethyl]phenyl]-3,5-difluorophenylcarboxamide; **LY451395**, [(2R)-2-[4-[4-[2-[(methylsulfonyl)amino]ethyl]phenyl]propyl][(1-methylethyl)sulfonyl]-amine.

Acknowledgments. The authors would like to thank P. Baumbarger, D. Calligaro, M. Gates, M. Mahlhauser, D. McCarty, E. Suida, M. A. Ward, and V. Vasudevan for help with some of the experimental data presented in this review.

Conflict of interest statement. The authors are employed by Eli Lilly & Co.

REFERENCES

1. Altar CA, Boylan CB, Fritsche M, et al. Efficacy of brain-derived neurotropic factor and neurotrohin-3 on neurochemical and behavioral deficits associated with parial nigrostrial dopamine lesions. *J Neurochem* 1994; 63:1021–1032.
2. Altar CA, Boylan CB, Jackson C, et al. Brain-derived neurotrophic factor augments rotational behavior and nigrostriatal dopamine turnover *in vivo*. *Proc Natl Acad Sci USA* 1992;89:11347–11351.
3. Bai F, Bergeron M, Nelson DL. Chronic AMPA receptor potentiator (LY451646) treatment increases cell proliferation in adult rat hippocampus. *Neuropharmacology* 2003;44:1013–1021.
4. Baumbarger PJ, Muhlhauser M, Yang CR, Nisenbaum ES. LY392098, a novel AMPA receptor potentiator: Electrophysiological studies in prefrontal cortical neurons. *Neuropharmacology* 2001;40:992–1002.
5. Baumbarger P, Muhlhauser M, Zhai J, Yang CR, Nisenbaum ES. Positive modulation of α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors in prefrontal cortical neurons by a novel allosteric potentiator. *J Pharmacol Exp Ther* 2001;298:86–102.
6. Beal M. F. Experimental models of Parkinson's disease. *Nat Rev Neurosci* 2001;2:326–332.
7. Benowitz LI, Routtenberg A. GAP-43: An intrinsic determinant of neuronal development and plasticity. *Trends Neurosci* 1997;20:84–91.
8. Bezard E, Dovero S, Bioulac B, Gross CE. Effects of different schedules of MPTP administration on dopaminergic neurodegeneration in mice. *Exp Neurol* 1997;148:288–292.
9. Bezard E, Dovero S, Bioulac B and Gross CE. Kinetics of nigral degeneration in a chronic model of MPTP-treated mice. *Neurosci Lett* 1997;234:47–50.
10. Brooks DJ, Frey KA, Marek KL et al. Assessment of neuroimaging techniques as biomarkers of the progression of Parkinson's disease. *Exp Neurol* 2003;184(Suppl 1):S68–S79.
11. Collingridge GL, Lester RAJ. Excitatory amino acid receptors in the vertebrate central nervous system. *Pharmacol Rev* 1989;40:143–210.
12. Coyle JT. The glutamatergic dysfunction hypothesis for schizophrenia. *Harvard Rev Psych* 1996;3(5): 241–253.
13. Davis GC, Williams AC, Markey SP, et al. Chronic Parkinsonism secondary to intravenous injection of meperidine analogues. *Psychiatry Res* 1979;1:249–254.
14. Dawson DA, Wadsworth G, Palmer AM. A comparative assessment of the efficacy and side-effect liability of neuroprotective compounds in experimental stroke. *Brain Res* 2001;892:344–350.
15. Dawson TM, Dawson VL, Neuroprotective and neurorestorative strategies for Parkinson's disease. *Nat Neurosci* 2002;5(Suppl):1058–1061.
16. Dicou E, Rangon CM, Guimiot F, Spedding M, Gressens P. Positive allosteric modulators of AMPA receptors are neuroprotective against lesions induced by an NMDA agonist in neonatal mouse brain. *Brain Res* 2003;970(1–2):221–225.
17. Gash DM, Zhang Z, Ovadia A, et al. Functional recovery in parkinsonian monkeys treated with GDNF. *Nature* 1996;380:252–255.
18. Gates M, Ogden A and Bleakman D. Pharmacological effects of AMPA receptor potentiators LY392098 and LY404187 on rat neuronal AMPA receptors *in vitro*. *Neuropharmacology* 2001;40:984–991.
19. Gill SS, Patel NK, Hotton, et al. Direct brain infusion of glial cell line-derived neurotrophic factor in Parkinson disease. *Nat Med* 2003;9:589–595.
20. Granger R, Deadwyler S, Davis M, et al. Facilitation of glutamate receptors reverse age-associated memory impairment in rats. *Synapse* 1996;22(4):332–337.
21. Hampson RE, Rogers G, Lynch G, Deadwyler SA. Facilitative effects of the AMPAKINE CX516 on short-term memory in rats — enhancement of delayed-nonmatch-to-sample performance. *J Neurosci* 1998;18(7): 2740–2747.
22. Hampson RE, Rogers G, Lynch G, Deadwyler SA. Facilitative effects of the AMPAKINE CX516 on short-term memory in rats — correlations with hippocampal neuronal activity. *J Neurosci* 1998;18(7):2748–2763.
23. Hayashi T, Umemori H, Mishina M, Yamamoto T. The AMPA receptor interacts with and signals through the protein tyrosine kinase Lyn. *Nature* 1999;397:72–76.
24. Hess US, Whalen SP, Sandoval LM, Lynch G, Gall CM. Ampakines reduce methamphetamine-driven rotation and activate neocortex in a regionally selective fashion. *Neuroscience* 2003;121(2):509–521.
25. Hume RI, Dingledine R, Heinemann SF. Identification of a site in glutamate receptor subunits that controls calcium permeability. *Science* 1991;253:1028–1031.
26. Langston JW, Ballard P, Tetrud JW, et al. Chronic parkinsonism in humans due to a product of meperidine-analog synthesis. *Science* 1983;219:979.
27. Lauterborn JC, Lynch G, Vanderklish P, Arai A, Gall CM. Positive modulation of AMPA receptors increases neurotrophin expression by hippocampal and cortical neurons. *J Neurosci* 2000;20:8–21.

28. Legutko B, Li X, Skolnick P. Regulation of BDNF expression in primary neuron culture by LY392098, a novel AMPA receptor potentiator. *Neuropharmacology* 2001;40:1019–1027.
29. Li X, Tizzano JP, Griffey K, Clay M, Lindstrom T, Skolnick P. Antidepressant-like actions of an AMPA receptor potentiator (LY392098). *Neuropharmacology* 2001;40:1028–1033.
30. Li X, Witkin JM, Need AB, Skolnick P. Enhancement of antidepressant potency by a potentiator of AMPA receptors. *Cell Mol Neurobiol* 2003;23(3):419–430.
31. Lynch G, Granger R, Ambrosingerson J, Davis CM, Kessler M, Schehr R. Evidence that a positive modulator of AMPA-type glutamate receptors improves delayed recall in aged humans. *Exp Neurol* 1997;145(1): 89–92.
32. Lynch G. AMPA receptor modulators as cognitive enhancers. *Curr Opin Pharmacol* 2004;4:4–11.
33. Mackowiak M, O'Neill MJ, Hicks CA, Bleakman D, Skolnick P. An AMPA receptor potentiator modulates the expression of BDNF: An *in vivo* study. *Neuropharmacology* 2002;43:1–10.
34. Mayer ML, Armstrong N. Structure and function of glutamate receptor ion channels. *Annu Rev Physiol* 2004;66:161–181.
35. Miu P, Jarvie KR, Radhakrishnan V, et al. Novel AMPA receptor potentiators LY392098 and LY404187: Effects on recombinant human AMPA receptors *in vitro*. *Neuropharmacology* 2001;40:976–983.
36. Morris RGM, Anderson E, Lynch GS, Baudry M. Selective impairment of learning and blockade of long-term potentiation by an N-methyl-D-aspartate receptor antagonist, AP5. *Nature* 1986;319:774–776.
37. Murray TK, Whalley K, Robinson CS, et al. LY503430, a novel α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor potentiator with functional, neuroprotective and neurotrophic effects in rodent models of Parkinson's disease. *J Pharmacol Exp Ther* 2003;306:752–762.
38. Nakajima, K., Hida, H., Shimano, Y. et al. GDNF is a major component of trophic activity in DA-depleted striatum for survival and neurite extension of DAergic neurons. *Brain Res* 2001;916:76–84.
39. Olanow CW, et al. A double-blind controlled trial of bilateral fetal nigral transplantation in Parkinson's disease. *Ann Neurol* 2003;54; 403–414.
40. O'Neill MJ, Lees KR. In: Danysz W, Lodge D, Parsons CG, Eds. *Ionotropic glutamate receptors as therapeutic targets*. Tennessee: F. P. Graham Publishing Co., 2002;403–447.
41. O'Neill MJ, Bleakman D, Zimmerman DM, Nisenbaum ES. AMPA potentiators for the treatment of CNS disorders. Current Drug Targets. *CNS Neurol Disord* 2004;3:181–194.
42. O'Neill MJ, Murray TK, Whalley K, et al. Neurotrophic actions of the novel AMPA receptor potentiator, LY404187, in rodent models of Parkinson's disease. *Eur J Pharmacol* 2004;486(2):163–174.
43. O'Neill MJ, Siemers ER. Pharmacological approaches to disease modifying therapies in Parkinson's disease. *Expert Rev Neurother* 2002;2:89–104.
44. Ornstein PL, Zimmerman DM, Arnold B, et al. Biarylpropylsulfonamides as novel, potent potentiators of α-amino-3-(5-methyl-3-hydroxyisoxazol-4-yl)- propanoic acid (AMPA) receptors. *J Med Chem* 2000;43: 4354–4358.
45. Parsons C, Danysz W, Lodge D. Chapter 1, Introduction to glutamate receptors, their function and pharmacology; In: Danysz W, Lodge D, Parsons CG, Eds. *Ionotropic glutamate receptors as therapeutic targets*. Tennessee: F. P. Graham Publishing Co., 2002;1–30.
46. Petroske E, Meredith GE, Callen S, Totterdell S, Lau Y-S. Mouse model of Parkinsonism: A comparison between subacute MPTP and chronic MPTP/probenecid treatment. *Neuroscience* 2001;106(3):589–601.
47. Quirk JC, Nisenbaum ES. LY404187: A novel positive allosteric modulator of AMPA receptors. *CNS Drug Rev* 2002;8:255–282.
48. Quirk JC, Nisenbaum ES. Multiple molecular determinants for allosteric modulation of alternatively spliced AMPA receptors. *J Neurosci* 2003;23(34):10953–10962.
49. Ravina BM, Fagan SC, Hart RG et al. Neuroprotective agents for clinical trials in Parkinson's disease: A systematic assessment. *Neurology* 2003;60:1234–1240.
50. Rosenbald C, Kirik D, Bjorklund A. Sequential administration of GDNF into the substantia nigra and straitum promotes dopamine neuron survival and axonal sprouting but not striatal reinnervation or functional recovery in the partial 6-OHDA lesion model. *Exp Neurol* 2000;161:503–516.
51. Skolnick P, Legutko B, Li X, Bymaster FP. Current perspectives on the development of non-biogenic amine-based antidepressants. *Pharmacol Res* 2001;43:411–423.
52. Speciale SG. MPTP: Insights into parkinsonian neurodegeneration. *Neurotoxicol Teratol* 2002;24:607–620.
53. Staubli U, Perez Y, Xu F, et al. Centrally activte modulators of glutamte receptors facilitate the induction of long-term potentiation *in vivo*. *Proc Natl Acad Sci USA* 1994;91(23):11158–11162.
54. Staubli U, Rogers G, Lynch G. Facilitation of glutamate receptors enhances memory. *Proc Natl Acad Sci USA* 1994;91(2):777–781.
55. Tamminga CA. Schizophrenia and glutamatergic transmission. *Crit Rev Neurobiol* 1998;12(1–2):21–36.

56. Tsai GC, Coyle JT. Glutamatergic mechanisms in schizophrenia. *Annu Rev Pharmacol Toxicol* 2002;42: 165–179.
57. Whone AL, Watts RL, Stossl AJ, et al. Slower progression of Parkinson's disease with ropinirole versus levodopa: The REAL-PET study. *Ann Neurol* 2003;54(1):93–101.
58. Wollmuth LP, Sobolevsky AI. Structure and gating of the glutamate receptor ion channel. *Trends Neurosci* 2004;27(6):321–328.
59. Wu X, Zhu D, Jiang X. et al. AMPA protects cultured neurons against glutamate excitotoxicity through a phosphatidylinositol 3-kinase-dependent activation in extracellular signal-regulated kinase to upregulate BDNF gene expression. *J Neurochem* 2004;90:807–818.
60. Vandergriff J, Huff K, Bond A, Lodge D. Potentiation of responses to AMPA on central neurones by LY392098 and LY404187 *in vivo*. *Neuropharmacology* 2001;40:1003–1009.
61. Yamada KA. Therapeutic potential of positive AMPA receptor modulators for the treatment of neurological disease. *Exp Opin Invest Drugs* 1998;323; 1361–1366.
62. Zigmond MJ, Stricker EM. Animal models of Parkinsonism using selective neurotoxins: Clinical and basic implications. *Int Rev Neurobiol* 1989;31:1–79.