LY503430, a novel AMPA receptor potentiator with functional, neuroprotective and neurotrophic effects in rodent models of Parkinson’s disease.

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Non-standard Abbreviations: 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

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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 firstly characterized a novel AMPA receptor potentiator, LY503430, on recombinant human GLUA1-4 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 sub-micromolar concentrations of 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. 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-hyrdroxypamine (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 increases in growth associated protein-43 (GAP-43) expression in the striatum. Therefore, we propose
that AMPA receptor potentiators offer the potential of a new disease modifying therapy for Parkinson’s disease.

Parkinson’s disease (PD) is a movement disorder resulting from neurodegeneration of the basal ganglia, the most prominent pathological change in Parkinsonian brains being the loss of dopaminergic innervation from the substantia nigra (SN) to the caudate and putamen of the corpus striatum. There are several available therapies to treat the symptoms (i.e. replacement of dopamine), but none halt or even slow the progression of the disease (O’Neill and Siemers, 2002). The exact mechanism of Lewy body formation and subsequent nigral cell death and the role played by environmental and genetic factor remains to be elucidated. However, it is clear that agents that halt the progression or help repair the damage are urgently required (O’Neill and Siemers, 2002).

With this in mind the actions of neurotrophins in Parkinson’s disease have recently been evaluated (Bradford et al., 1999). Many investigators have reported that glial derived growth factor (GDNF) promotes dopamine neuron survival (Tomac et al., 1995; Gash et al., 1996; Rosenbald et al., 2000), while other studies have shown that brain derived growth factor (BDNF) can protect against behavioural, biochemical and immunocytochemical changes after nigrostriatal dopamine lesions (Altar et al., 1994, 1994; Klein et al., 1999). A major drawback of growth factors is their large size and therefore the need to administer these molecules directly into the brain. An alternative and perhaps more clinically relevant approach would be to use a small molecule that could up-regulate neurotrophin expression in the brain. Of direct relevance therefore is the recent report that AMPA receptors interact with and signal.
through the protein tyrosine kinase, Lyn (Hayahi et al., 1999). As a result, the
mitogen-activated protein kinase (MAPK) pathway is activated and the expression of
BDNF is increased (Hayashi et al., 1999).

Following the discovery that cyclothiazide and IDRA 21 could potentiate
AMPA receptor activity, more potent and selective AMPA receptor potentiators have
been discovered (Baumbarger et al., 2001b; Parsons et al., 2002; Ornstein et al.,
2000). All of these compounds allosterically regulate AMPA receptor activity at least
in part by suppressing the desensitization process of AMPA receptors. As a result
these molecules can enhance calcium flux in HEK293 cells transfected with
recombinant human GLU_A1-4 subunits (Miu et al., 2001) and increase AMPA-evoked
responses on native neurons in vitro (Baumbarger et al., 2001a,b; Gates et al., 2001)
and in vivo (Vandergriff et al., 2001), enhance LTP and are active in various
cognitive models (Staubli et al., 1994; Hampson et al., 1998a,b; Quirk and
Nisenbaum, 2002). In addition it has also been shown that AMPA potentiators such
as CX-546 (Lauterborn et al., 200), LY392098 (Legutko et al., 2001) and LY404187
(Mackowiak et al., 2002) increase BDNF levels in vitro and in vivo. Based on these
observations, we hypothesised that an AMPA potentiator would protect against
nigrostriatal degeneration.

In the present studies we have evaluated the effects of a novel AMPA receptor
potentiator, LY503430 on glutamate responses in HEK293 cells transfected with
human GLU_A1, GLU_A2, GLU_A3, or GLU_A4 AMPA receptor subunits and on glutamate
responses in hippocampal, Purkinje, cortical and substantia nigra neurons in vitro.
We also present results showing that this novel AMPA potentiator, provided
significant neuroprotection in a mouse MPTP and two rat 6-OHDA models of
Parkinson’s disease. Furthermore in the unilateral 6-OHDA model, functional and
histological protection were maintained when LY503430 treatment was delayed for up to 14 days suggesting a trophic action. These data suggest that LY503430 could protect and potentially reverse the progression of Parkinson’s disease.
Methods

Studies on recombinant iGlu receptors expressed in HEK293 cells: 96-well plates containing confluent monolayers of HEK293 cells stably expressing human AMPA receptors were prepared. Cells were incubated in buffer solution (10 mM glucose, 138 mM sodium chloride, 1 mM magnesium chloride, 5mM potassium chloride, 5 mM calcium chloride, 10 mM N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid, to pH 7.1-7.3) containing 20 µM Fluo3-AM dye (obtained from Molecular Probes Inc., Eugene, Oregon, USA) for 60 min. Cells were washed in buffer solution and fluorescence measurements were made using a FLIPR (Molecular Devices, Sunnyvale CA, USA) that indicated changes in fluorescence upon influx of calcium into cells upon stimulation by glutamate (100 µM) in the presence of cyclothiazide (100 µM) or compound. Compound applications preceded glutamate additions by 5 min and fluorescent measurements were made immediately prior to addition of glutamate and 3 min following glutamate addition. Data are expressed as EC50 values determined from maximum responses observed for LY503430 on each cell line in the presence of glutamate (100 µM).

AMPA receptor-mediated responses in acutely isolated substantia nigra and prefrontal cortical neurons:

Prefrontal cortical pyramidal neurons or substantia nigra dopamine neurons from young, (2-3 weeks old) male, Sprague-Dawley rats (14-22 days old) were acutely isolated from the prefrontal cortex or the midbrain, respectively using previously described procedures (Baumbarger et al., 2001b). Male Sprague-Dawley rats were deeply anesthetized with methoxyflurane and decapitated. Their brains were removed
rapidly from the skull and immersed in a cold (~2°C) NaHCO₃-buffered saline solution (concentrations in mM): NaCl 126.0, KCl 3.0, MgCl₂ 1.5, Na₂PO₄ 1.25, CaCl₂ 2.0, NaHCO₃ 26.0, glucose 10.0; pH = 7.4, osmolarity = 300 ± 5 mOsm/liter.

The brains were blocked and 400 µm thick coronal sections were cut through the rostrocaudal extent of the prefrontal cortex or midbrain using a Vibroslice (Campden Instruments, London, England). Slices then were incubated at room temperature (20–22°C) for 0.5–6 hr in a holding chamber containing the continuously oxygenated (95% O₂: 5% CO₂) NaHCO₃-buffered saline solution. Following the incubation period, slices were transferred to a glass petri dish containing a low Ca²⁺, N-[2-hydroxyethyl]piperazine-N-[2-ethanesulfonic acid] (HEPES)-buffered saline solution (concentrations in mM): NaHOCH₂CH₂SO₃ (Na isethionate) 140.0, KCl 2.0, MgCl₂ 4.0, CaCl₂ 0.1, glucose 23.0, HEPES 15.0; pH = 7.4, osmolarity = 300 ± 5 mOsm/liter and placed under a dissecting microscope. The prefrontal cortex or ventral midbrain from each hemisphere was dissected from the surrounding tissue.

The tissue was placed into a holding chamber containing protease Type XIV. (1 mg/ml; Sigma Chemical Co., St. Louis, MO) dissolved in a HEPES-buffered Hank’s balanced salt solution (HBSS #6136; Sigma Chemical Co., St. Louis, MO) maintained at 37°C and oxygenated (100% O₂), pH = 7.4, osmolarity = 300 ± 5 mOsm/liter. Following 30–40 min of incubation in the enzyme solution, the cortex was rinsed three times with the low Ca²⁺ HEPES-buffered saline solution and triturated using two fire-polished Pasteur pipettes having tips of decreasing diameter.

Prior to whole-cell recording, the cell suspension was placed into a 50 mm transparent plastic petri dish which was mounted onto the stage of an inverted microscope. Prefrontal cortical pyramidal (PFC) neurons were selected on the basis of their triangular somatic shape, soma size (~20-30 µm in diameter), and presence of
some apical and basal dendrites. Substantia nigra dopamine neurons were identified on the basis of their large soma size and multipolar morphology.

The whole-cell variant of the patch-clamp technique was used for recording current from acutely isolated PFC pyramidal neurons. Electrodes were pulled from borosilicate capillary tubing (Corning 7052, WPI Inc., Sarasota, FL) using a multistage puller (Sutter Instruments Inc., Novato CA). The electrodes were fire-polished using a microforge (Narishige Inc., Tokyo, Japan) prior to use. The internal electrode filling solution contained (concentrations in mM): N-methyl-D-glucamine, 160.0, MgCl₂ 4.0, HEPES 40.0, BAPTA 3.0, phosphocreatine 12.0, Na₂ATP 2.0, guanosine-5′-triphosphate (GTP) 0.2; pH was adjusted to 7.2 with KOH and osmolarity adjusted to 270–280 mOsm/liter. The extracellular solution contained (concentrations in mM): Na isethionate 140.0, KCl 1.0, BaCl₂ 5.0, MgCl₂ 1.0, HEPES 10.0, TTX 0.001; pH adjusted to 7.4 with NaOH 1.0 M; osmolarity adjusted to 300 ± 5 mOsm/liter with glucose.

Upon placing the recording electrode in the bath, offset potentials were corrected and electrode resistances ranged between 2 and 7 MΩ. Voltage-clamp recordings were made using an Axon Instruments 200B amplifier (Axon Instruments Inc., Foster City, CA). The membrane potential of cells was held at −80 mV. Currents were digitized and monitored with pCLAMP software version 8.0 (Axon Instruments Inc., Foster City, CA) running on a PC Pentium computer. A small amount of constant positive pressure (2-3 cm H₂O) was applied to the electrodes as they were advanced through the bath. After achieving the whole-cell configuration, series resistance was compensated (70-85%) and monitored periodically. All experiments were conducted at room temperature.
Application of drugs was accomplished using sixteen barrel pipette array made from small diameter (~ 600 µm) glass capillary tubing. Solutions were contained in 10 ml syringes and positioned approximately 12 inches above the recording chamber. Gravity-induced flow of each solution from the syringe to the corresponding barrel was controlled by electronic valves. The pipette array was positioned 100-200 µm from the cell prior to seal formation. The solutions from the drug array were changed (~100 ms) by altering the array position with a DC actuator (Newport Inc., Irvine, CA).

Concentration-response profiles for LY503430 and CX-516 were constructed by measuring the peak current amplitude during a 10 s coapplication of compound and AMPA (5 µM), calculating the percent increase relative to the AMPA alone response and plotting the data as a function of potentiator concentration. The plotted points then were fit with a logistic equation of the form,

\[
\text{Percent Potentiation} = \frac{E_{\text{max}}}{1 + ([\text{AMPA potentiator}] / EC_{50})^n}
\]

Where the maximal percent potentiation is relative to the current evoked by glutamate alone, EC_{50} is the concentration equal to 50% of the maximally effective concentration, and n is the Hill coefficient. The best fit was chosen using the Marquardt-Levenberg algorithm. Average EC_{50} and E_{max} values were determined and reported as mean ± standard error of the mean (sem).

**Responses of hippocampal neurones to iontophoretic application of AMPA and NMDA in vivo:** The principles of the iontophoresis method are well established and described previously (Vandergriff et al., 2001). Briefly, male Sprague-Dawley rats
(300-400g) were anaesthetized with chloral hydrate 400 mg/kg i.p. initially and supplemental doses intermittently to maintain surgical anaesthesia. The rats were mounted in a stereotaxic frame and one or two small craniotomies were performed to allow placement of stimulating and recording electrodes. For the hippocampal iontophoretic studies, five barrel glass microelectrodes (tip diameter 5-8µm) were positioned in the CA1 region of the hippocampus. The center recording barrel contained 2M NaCl, outer barrels contained AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; 5 mM in 195 mM NaCl; pH 7.4), NMDA (N-methyl-D-aspartic acid; 20 mM in 180 mM NaCl; pH 7.1) and 2 M NaCl, the latter being used for current balancing. Hippocampal CA1 pyramidal cells were located and identified by their stereotaxic co-ordinates (Bregma –4.2, lateral 2.4, 1.8-2.4 mm below pial surface) and characteristic extracellular action potential. Excitation of CA1 neurones was achieved by the alternate and electronically timed electrophoretic ejection of AMPA and NMDA with currents and durations of ejection (normally 0-10 nA and 20-30 s) adjusted to produce near equal and submaximal increases in firing rate. Once stable responses were maintained for a period of at least 10 min, the test drug was administered via a lateral tail vein cannula (Vandergriff et al., 2001). Cumulative dose-response curves to CX-516 and LY503430 were prepared by recording responses to increasing drug doses in logarithmic steps. Raw data and firing data were recorded on a DAT analyzer for subsequent analysis. Changes were calculated as percentage of control firing rates and expressed as the mean ± S.E.M.

**MPTP neurotoxicity in mice:** Male C57Bl6J mice (Harlan UK Ltd, Oxford, UK) weighing 20-25g were used. They were housed in groups of ten mice per cage under
a 12-h light/12-h dark cycle (lights on 7.00 a.m. - 7.00 p.m.) with food and water available ad libitum. LY503430 was administered at 0.5 mg/kg s.c for 11 days. On day 8 the animals received 4 x 20 mg/kg MPTP at 2 hr intervals.

**6-OHDA studies in rats:** Male Sprague Dawley rats (Harlan UK Ltd, Oxford, UK) weighing 280-320g 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.

**Substantia nigra lesions:** Unilateral lesions of the left substantia nigra were made using 4µg (free base) 6-OHDA in 1.8µl 0.02% ascorbic acid infused stereotaxically into the left substantia nigra (SN)- co-ordinates from bregma according to the atlas of Paxinos and Watson (1986) were AP: -4.8mm, L: +1.9mm, V: -8.0mm (from skull surface at bregma) and toothbar –3.7mm. 6-OHDA was infused at a rate of 0.3µl/min, followed by 2 min equilibration time, with the needle remaining in place (Murray et al., 2002). Sham-operated rats received identical surgery but 1.8µl 0.02% ascorbic acid (6-OHDA vehicle) was infused.

**Striatal lesions:** Unilateral lesions of the right striatum were made using 10µg (free base) 6-OHDA in 2.57µl 0.02% ascorbic acid in 0.9% saline infused stereotaxically into the right striatum - co-ordinates from bregma were AP: 0.7mm, L: -2.3mm, V: -6.0mm (from skull surface at bregma) and toothbar – 3.3mm. The infusion was made over a period of 4 min at a rate of 0.643µl/min, followed by 4 min equilibration time, with the needle remaining in place (Murray et al., 2002).
**Drug studies:** For all s.c. studies LY503430 was dissolved in 12.5% β-cyclodextrin and sonicated prior to administration. For all oral studies LY503430 was dissolved in 1% sodium carboxymethylcellulose/0.25%Tween 80/water vehicle.

For nigral lesion studies, LY503430 was administered for either 14 days at 0.1 or 0.5 mg/kg s.c. in initial studies or 10 days at 0.05, 0.1, 0.2 or 0.5 mg/kg p.o to complete full oral dose response starting 1 day after 6-OHDA lesion. Compounds were administered twice daily on week-days and once a day at week-ends. In additional studies treatment with LY503430 was delayed until 3, 6 or 14 days after 6-OHDA infusion. For studies using striatal lesion model LY503430 was administered for 28 days at 0.5 mg/kg s.c. starting 1 day after 6-OHDA lesion.

**Behavioural Assessment using rotometers:** Twenty-four hours after the final drug treatment the animals were placed in automated rotometers (Med. Associates Ltd.). 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 rotations. The animals were tested for baseline rotations 24 hr after the final drug treatment. On the next day the animals were retested in the presence of apomorphine (0.25 mg/kg s.c.) or amphetamine (5 mg/kg i.p.) to evaluate the effects of drug treatment on stimulant-induced rotations (this is a measure of functional neuroprotection). Data was expressed as asymmetry scores (the difference between the number of contraversive and ipsiversive rotations).
Neurochemical measurements of dopamine and metabolites: The left and right striata were dissected, weighed and homogenised in 2 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. 2 µl of the supernatant was then assayed by HPLC with EC detection. All analyses were performed on a Luna 5 C18 column (25 cm x 2 mm) at a flow rate of 200µl/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 pre-cleaned 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 was collected on a Waters Millennium chromatography data system. Dopamine, DOPAC and HVA concentrations in the samples were calculated by comparison with calibration curves constructed from pure reference standards.

Histological Analysis: After behavioural testing the animals were given an overdose of anaesthetic, the thorax opened and perfused with 30ml of saline followed by 30ml of 10% buffered formalin via the left ventricle or vena cava. The brains were removed, cut into 2 x 6 mm segments using a rodent brain matrix, processed and embedded in paraffin wax. 8 µm coronal sections were taken on a sledge microtome (Murray et al., 2001).
Tyrosine hydroxylase, GAP-43 and BDNF immunocytochemistry: Briefly, the sections were deparaffinised and rehydrated and endogenous peroxidase was quenched with 0.3% H$_2$O$_2$ for 30 min. The slides were placed in pepsin (0.2g Sigma-p-7000 pepsin in 50ml 0.01M HCl) for 30 min, washed and non-specific binding was blocked with 1.5% normal goat serum (Vectastain rabbit IgG ABC kit). This was followed by application of the primary rabbit polyclonal anti-tyrosine hydroxylase antibody (Chemicon AB152 incubated for 18 hours at room temperature), the secondary biotinylated antibody (Vectastain rabbit IgG ABC kit for 30 min) and the HRP conjugate (Vectastain rabbit IgG ABC kit for 30 min). Visualisation was carried out using 3, 3'-diaminobenzidine (Vector SK-4100) as a chromogen. The slides were coverslipped using DPX mountant. Adjacent sections from the oral efficacy studies were stained in a similar way using a primary antibody (Chemicon AB5220, 1:500 dilution) to growth associated protein 43 (GAP-43) and/or a primary antibody (Santa Cruz Biotechnology SC-546 rabbit polyclonal IgG 1:100 dilution) to BDNF.

Image Analysis: After TH-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 (Murray et al., 2002). Sections of the substantia nigra at -5.00 mm caudal to bregma in the rat (Paxinos and Watson, 1986) and at -3.08 mm caudal to bregma in mouse (Paxinos and Watson, 1997) were also stained and the number of intact TH positive cells in left and right substantia nigra were counted at x25 magnification. The same image analysis system
was used to quantify the GAP-43 and BDNF immunoreactivity. The mean grey intensity of the intact and lesioned striatum was calculated and data was then expressed as a percentage change in GAP-43 immunoreactivity between the intact and lesioned hemisphere. The number of BDNF positive immunoreactive cells per field were counted in the substantia nigra, striatum, hippocampus (CA1, CA3, dentate gyrus) and in the cortex.

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


Results

1. POTENCY AND SELECTIVITY OF LY503430.

1.1 Effects on recombinant GLU_{A1-4} receptors expressed in HEK293 cells:

The AMPA potentiator used for this investigation was the biarylpropylsulfonamide, LY503430 (Fig. 1). Sub-micromolar concentrations of LY503430 enhanced glutamate-induced calcium influx into HEK293 cells transfected with human GLU_{A1}, GLU_{A2}, GLU_{A3}, or GLU_{A4} AMPA receptors. The potency and efficacy of potentiation by LY503430 was highly dependent on receptor subtype and splice variant. The rank order of potency of LY503430 was GLU_{A2} > GLU_{A4} > GLU_{A1} > GLU_{A3} and at all subunits the compound was considerably more potent on the “flip” splice variants.

1.2. Effects LY503430 and CX-516 substantia nigra dopamine neurons and prefrontal cortical neurons in vitro:

The potency and efficacy of LY503430 (0.03-10 µM) and CX-516 (0.3-3.0 mM) on the AMPA-evoked responses of substantia nigra dopamine neurons was evaluated by recording the inward current elicited in response to application of AMPA (5 µM, 10 s duration; holding potential = -80 mV) alone and in the presence of each compound. Preliminary experiments demonstrated that this concentration of AMPA was equal to 30% of the maximal response (EC_{30}). The relatively small responses recorded in the presence of AMPA reflect only the desensitized component of the total AMPA response; the peak component is not detectable because of the relatively slow solution switching speed (~100 ms) of the actuator used in these experiments. At all concentrations tested, application of LY503430 alone had no effect on the holding current (Fig. 2a). However, when applied in the presence of AMPA, LY503430
potentiated the evoked current in a concentration-dependent manner (Fig. 2 a,b). As previously reported for biarylsulfonamides (Baumbarger et al., 2001b), the potentiated response displayed a marked time-dependence such that a steady-state level was never achieved during the 10 s AMPA stimulus or even if applications were prolonged for up to 120 s. Because of this property, the data were expressed as a percent change in peak amplitude from that of the glutamate response alone and plotted as a function of compound concentration. As such, the values for potency and efficacy are estimates. Comparison of the concentration-response profiles for LY503430 and CX-516 revealed that LY503430 was more potent (EC50 = 2.6 ±0.3 µM) and efficacious (Emax = 181.3 ± 40.0-fold increase, n=8) than CX-516 (EC50 = >3 mM) and efficacy (Emax = 11.2 ± 2.4-fold increase; n=9). Similar differences in potency and efficacy of potentiation of prefrontal cortical neurons (Fig. 2c) were found for LY503430 (EC50 = 3.3 ± 0.8 µM; Emax = 86.7 ± 14.3-fold increase, n=14) and CX-516 (EC50 = 3.7 ± 1.3 mM; Emax = 3.8 ± 1.6-fold increase, n=6).

1.3 Cross reactivity on other glutamate receptor subtypes:
LY503430 had no discernible effects on kainate-mediated currents in HEK293 cells transfected with GLU K5, GLU K6 or GLU K6/KA2 at concentrations up to 10 µM or on responses of rat cortical and hippocampal neurons to NMDA and to kainate in the presence of GYKI53655 (a selective AMPA antagonist). On rat DRG neurons LY503430 (3µM) slightly inhibited responses to kainate (Fig. 1). In addition, ligand binding studies indicated that LY503430 did not bind to other neurotransmitter (adrenergic, nicotinic, muscarinic, serotonergic, dopaminergic, etc) receptors (data not shown).
1.4. Effects of LY503430 on AMPA responses of rat hippocampal neurons in vivo:

Systemic administration of LY503430 (0.01-10 µg/kg, i.v.) enhanced responses of hippocampal neurons to iontophoretic AMPA in a dose-dependent manner (Fig 2d). There was a smaller increase in responses to NMDA. The dose-response curves for LY503430 are compared to that of CX-516 (Fig. 2d). A dose of 0.1 µg/kg i.v. of LY503430 was sufficient to produce a selective increase in hippocampal responses to AMPA, confirming that LY503430, crosses the blood brain barrier and has central actions.

2. NEUROPROTECTIVE EFFECTS OF LY503430 IN RODENT MODELS OF PARKINSON’S DISEASE.

2.1. Prevention of MPTP induced neurotoxicity in mice:

LY503430, administered for 11 days at 0.5 mg/kg s.c. prevented the loss of tryosine hydroxylase immunoreactivity (TH-IR) in the striatum (Fig. 3a) and substantia nigra (Fig. 3b) in MPTP-treated mice.

2.2. Functional and histological protection after infusion of 6-OHDA into the striatum in rats:

In initial studies we found that 10 µg of 6-OHDA infused unilaterally into the striatum produces a slow, partial retrograde degeneration of the cell bodies in the substantia nigra resulting in an approximate 50% loss in tyrosine hydroxylase positive cells at 4 weeks and in marked ipsiversive rotations in response to amphetamine (5 mg/kg). Using this model, we found that 28 days treatment with LY503430 (0.5 mg/kg s.c.) attenuated amphetamine-induced ipsiversive rotations.
and provided significant protection against the loss of TH positive nigral cell bodies (Fig 4b and c).

It should be pointed out that in these and the subsequent experiments, the LY503430 treatment was stopped 1-2 days before the behavioural tests and in separate experiments acute dosing of LY503430 was without effect on turning behaviour in previously lesioned control animals.

2.3. Functional and histological protection after infusion of 6-OHDA into the substantia nigra in rats:

In initial studies, 4 µg of 6-OHDA infused into the substantia nigra produced a loss of cell bodies over the next 4 days and striatal terminals over the next 5-6 days, resulting in an 85-90% loss in nigra cell bodies, 80-90% loss in of TH-IR in the dorsal striatum and 50-60% loss in TH-IR in the ventral striatum. 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 1 day after 6-OHDA) on functional outcome at 12 days and histological outcome at 13 days after 6-OHDA. Results of the first experiment indicated that both the 0.2 and 0.5 mg/kg oral doses of LY503430 prevented apomorphine-induced rotations (Fig 5a) and provided significant protection in the dorsal and ventral striatum (Fig 5b and c). These effects were accompanied, by only a modest effect on the number of TH positive cells in the substantia nigra.

Additional experiments (using the same protocol) indicated that 0.1 (Fig 6A and B) and 0.05 mg/kg (Fig 6C and D) of LY503430 attenuated apomorphine-induced rotations and protected against the loss of DAergic terminals in the dorsal striatum to a reduced extent (this is summarised in Table 1).
In a separate experiment, we measured levels of dopamine and its metabolites and found that the imbalances caused by nigral infusion of 6-OHDA were reduced by the above treatment with LY503430. Thus, LY503430 at 0.5 mg/kg reduced lesion-induced depletions in dopamine (dopamine levels in the lesioned hemisphere were: 9424 ± 1002 in sham operated, 807 ± 220 in vehicle–treated animals, while values in LY503430–treated animals were 2775 ± 343). Thus, oral administration of LY503430 provided functional and histological evidence of neuroprotection in a dose-dependent manner.

3. NEUROTROPHIC EFFECTS OF LY503430 IN RODENT MODELS OF PARKINSON’S DISEASE.

3.1. Effects on of LY503430 on GAP-43 immunoreactivity in the striatum:

The large functional effect and robust protection of DAergic terminals in the striatum with only minimal protection of cell bodies in the nigra at the highest dose suggested that LY503430 was having a trophic action after infusion of 6-OHDA into the substantia nigra. While assessing TH levels in the striatum, we stained adjacent sections for BDNF and GAP-43. Results indicated that LY503430 provided a dose-dependent increase in GAP-43, but not BDNF in the lesioned striatum (Fig 7). We were also not able to detect any changes in BDNF in the striatum or in the hippocampal regions, but did observe an increase in the substantia nigra (Fig 8). This increase may provide trophic actions on the cell bodies to enhance neurite outgrowth in striatal terminals.

3.2 Effects of delayed treatment with LY503430 after unilateral nigral lesion:
To distinguish between a neurotrophic and a neuroprotective role, we varied the start of treatment with LY503430 following nigral infusion of 6OHDA. The level of striatal tyrosine hydroxylase staining was similar whether administration was initiated 1, 3, 6 or 14 days after infusion of 6-OHDA; the data from three such independent experiments are illustrated in Fig 9. Even, when LY503430 treatment was initiated 1 hr before 6-OHDA, there was no improvement over administration at the later time points. Thus, treatment with LY503430 at any time point resulted in 30% TH immunoreactivity in the dorsal striatum, in comparison with 10% in vehicle treated animals. In parallel with the histology, the behavioural scores were also improved by delayed treatment with LY503430 (for example when treatment was initiated at 6 days after 6-OHDA the rotational values were 112 ± 11 rotations in 40 min for vehicle-treated animals and 55 ± 16 rotations in 40 min for LY503430-treated animals). This apparent neurotrophic effect was also not due simply to upregulation of TH per se, as there was no effect in control animals treated with LY503430 (Fig. 9) and there were no effects of the compound acutely on turning behaviour in lesioned animals. Indeed in all 195 animals chronically treated with LY503430 there were no overt changes in their behaviour.
Discussion

In the present study, we have demonstrated that a novel, potent, selective and systemically active AMPA receptor potentiator provided neuroprotective actions in three rodent models of Parkinson’s disease. The compound also increased GAP-43 expression in the lesioned striatum and reduced the 6-OHDA-induced effects on turning behavior and on striatal DAergic innervation. LY503430 is a new class of compound with potential utility for providing neuroprotection and neuronal repair in Parkinson’s disease.

LY503430 AS A SELECTIVE POTENTIATOR OF AMPA RECEPTORS

This novel biarylpropylsulfonamide selectively potentiated responses of human recombinant and rat native AMPA receptors. The results indicate that LY503430 is more selective for the “flip” splice variants and in particular for hGLUA2 with an EC$_{50}$ value of 33nM, whereas the EC$_{50}$ value was 2.25µM on the ‘flop’ variant. AMPA receptor currents in isolated substantia nigra, striatal, hippocampal and prefrontal cortical neurones were also potentiated with EC$_{50}$ values of 100 nM to 7 µM, illustrating the heterogeneity across central neurones. In contrast, functional NMDA, GLU$_{K5}$, and GLU$_{K6}$, GLU$_{K6/KA2}$ receptor activities in hippocampal, cortical, dorsal root ganglion (DRG) and transfected HEK 293 cells, as previously described with other AMPA potentiators (Gates et al., 2001; Baumbarger et al., 2001a,b) were not affected by concentrations of 10-100 µM LY503430. In addition, LY503430 showed no binding affinity at 18 other different neurotransmitter receptors (D. Calligaro unpublished observations).
Systemic administration of LY503430 (0.01-10 µg/kg, i.v.) also enhanced responses of hippocampal neurons to AMPA in a dose-dependent manner suggesting reasonable blood-brain barrier permeation. The small increase in NMDA responses is likely to be due to enhancement of tonic AMPA receptor activity, causing depolarisation and relieving the Mg$^{2+}$ brake of NMDA receptor channels, as shown previously for other AMPA potentiators (Vandergriff et al., 2001). For comparison, the equivalent dose of CX-516 required to produce a 20% increase in AMPA-mediated responses was 1000 µg/kg, i.v.

**NEUROPROTECTIVE ACTIONS OF LY503430.**

The exact mechanisms of Lewy body formation and cell death in PD remains to be elucidated, but evidence suggests that it is a combination of oxidative stress, genetic (Scott et al., 1997) environmental factors (Tanner and Langston, 1990) and malfunction of ubiquitin-proteosome systems (McKnight et al., 2001). Familial PD is associated with mutations in Parkin or α-synuclein genes, but it is interesting that α-synuclein overexpressing mice have inclusion bodies, but no cell death (Masliah et al., 2000). Other recent studies have shown that the mitochondrial Complex I inhibitor, rotenone, can produce a slow progressive degeneration of dopaminergic neurones, with inclusion bodies in some rats (Betarbet et al. 2000). In the present studies we have used well established models with MPTP and 6-OHDA that produce cell loss and permanent dopamine depletion in vivo (Flint Beal et al., 2001). Our results indicated that LY503430 could protect whether we used systemic MPTP in mice, or infusion of 6-OHDA in the rat either into the cell body region, which produces a relatively rapid lesion or into the striatum, which produces a slow partial
lesion of the nigra. We counted the number of intact nihral cells at one stereotaxic level and further studies using stereological methods are required to provide conclusive evidence for neuroprotection. However, in most cases, we used both rotational behaviour and TH immunoreactivity in the same animals to demonstrate the improvement in outcome, which were also confirmed by measurement of dopamine and its metabolites in a separate experiment.

Many investigators, have reported that antioxidants, nitric oxide synthase inhibitors, anti-inflammatory agents, nicotine, immunophilins and related molecules can provide protection in these models (Korcyn and Nussbaum, 2002; O’Neill and Siemers, 2002). Our in-house comparisons indicate, however, that LY503430 provides superior protection to all these other pharmacological interventions. We have not evaluated central administration of growth factors, but, for example, GDNF is effective in both nigral and striatal 6-OHDA lesion models (Altar et al., 1992, 1994; Rosenbald et al., 2000). In addition in the current studies we observed an increased level of BDNF immunoreactivity in the substantia nigra after LY503430 treatment in the 6-OHDA lesioned animals.

NEUROTROPIC ACTIONS OF LY503430.

The finding that, after LY503430 treatment there was a greater increase in tyrosine hydroxylase staining in the terminal region relative to the cell body region in the nigro-striatal pathway, led us to propose a neurotrophic mode of action. Indeed, in this protocol we found an upregulation of the neurotrophic agent, GAP-43, in the
lesioned animals treated with LY503430. It is well established that GAP-43 (growth
associated protein) is implicated in formation of new synapses and neurite outgrowth
(Benowitz and Routtenberg, 1997; Namgung and Routtenberg, 2000). For example,
transgenic animals overexpressing GAP-43 exhibit increased sprouting and repair and
GAP-43 is up-regulated in the penumbra following ischaemic brain lesions.

The improvement in both functional and histological outcomes, even when
treatment was delayed until after the cell death process, provides direct evidence of a
neurotrophic effect. Because LY503430 has little effect on the number of surviving
nigral neurones but does increase the tyrosine hydroxylase staining in the striatum,
the improvement is largely confined to the terminal regions, which is consistent with
enhanced sprouting. The parallel improvement in the behavioural measure suggests
that this increase in tyrosine hydroxylase staining represents functional dopaminergic
synapse formation. Furthermore, since the degree of improvement is similar whether
the treatment is started immediately before, during or after the cell death process, this
neurotrophic effect appears to be the dominant feature of the drug’s action. In
support of this, treatment with LY503430 from days 1-5 post-lesioning was
ineffective, suggesting that whatever the mechanism of action it takes several days to
activate the effect. This is consistent with the downstream activation by AMPA
receptors of one or more signalling pathways (Lyn kinase, MAP kinase, etc.). Thus,
we hypothesise that in this way LY503430 has trophic actions via the remaining
nigral cell bodies and/or their striatal terminals to enhance DAergic sprouting.

MODE OF ACTION OF LY503430.

The exact mechanism of neuroprotection is not clear, but one mechanism may
be an up-regulation of trophic factors, in particular BDNF. In 1999, Hayashi and co-
workers reported that AMPA receptor potentiators can increase BDNF via the 
tyrosine kinase Lyn, which is physically associated with the AMPA receptor (Hayashi 
et al., 1999). Further studies have demonstrated that AMPA potentiators can increase 
BDNF expression in both cerebellar granule and hippocampal neurones (Legutko et 
al., 2001) and in astrocytic cultures (V. Lakics et al, unpublished data) \textit{in vitro}. In 
addition sub-chronic treatment with LY404187 was reported to increase TrkB and 
BDNF mRNA in the rat hippocampus \textit{in vivo} (Mackowiak et al., 2002). We did not 
observe any increases in BDNF using immunocytochemical methods in striatal slices 
at the end of the current efficacy studies. The lack of changes in the striatum may be 
because (1) the BDNF levels are too low here (2) immunocytochemistry is not of 
sufficient sensitivity (3) the 12-14 day end-points are too late and we missed the 
effect, (4) the BDNF is increased in other brain regions and (5) BDNF is not 
involved. However, we did observed an increase in BDNF levels in the substantia 
nigra after 0.5 mg/kg LY503430, suggesting it may play some role. It is also clear 
that both BDNF and GDNF can protect dopaminergic neurones \textit{in vitro} and \textit{in vivo} 
(Rosenbald et al., 2000) and BDNF plays a key role in synaptic plasticity (Kovalchuk 
et al., 2002; Messaoudi et al., 2002). We also observed a dose-dependent increase in 
GAP-43 in both the intact and lesioned striatum. The magnitude of the 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 cell culture systems and agents that 
increase GAP-43 in rat cerebral cortex accelerate functional recovery \textit{in vivo}. It seems 
likely that LY503430 signalling through the AMPA receptor can increase trophic 
factors (such as BDNF) and enhance sprouting of striatal terminals (in this case 
increased GAP-43).
CONCLUDING REMARKS.

In conclusion, we have demonstrated that LY503430 is a potent potentiator of recombinant human and rat native AMPA receptors in vitro and can also increase AMPA-evoked responses in vivo. The present results provide the first evidence that AMPA receptor potentiators can provide functional and histological protection in rodent models of PD. In addition these effects were maintained after delayed administration and accompanied by an increase in GAP-43 expression in the striatum provided tantalising evidence for neurotrophic action. AMPA potentiators have been shown to be effective in animal models of depression (Li et al., 2001; Skolnick et al., 2001), rodent models of cognition (Hampson et al., 1998a,b; Quirk and Nisenbaum, 2002; Staubli et al., 1994) and in some memory tests in aged humans (Lynch et al., 1997) and hence may be of additional benefit in PD patients by treating these concurrent symptoms. These results suggest that LY503430 or another related AMPA potentiator would be a suitable molecule to advance as a clinical candidate to reduce or halt and potentially reverse the degeneration observed in human Parkinson’s disease.
ACKNOWLEDGEMENTS

We would like to thank David Caligaro for valuable help profiling LY503430 across neurotransmitter receptor sub-types, Sandra Woodhouse for help with dopamine and metabolite measurements and Roger Moore for statistical assistance.
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potent potentiators of 2-amino-3- (5-methyl-3-hydroxyisoxazol-4-yl)- propanoic acid (AMPA) receptors. J. Med. Chem. 43: 4354-4358.


FOOTNOTES PAGE:

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Figure Legends

**Fig. 1.** The structure and pharmacological profile of the biarylpropylsulfonamide, LY503430. EC$_{50}$ values (nM) for calcium flux at recombinant human AMPA (GLU$_{A1-4}$ flip and flop) and kainate (GLU$_{K5}$, GLU$_{K6}$ and GLU$_{K6/KA2}$) receptor subtypes expressed in HEK293 cells. Responses on NMDA receptors in cortical and hippocampal neurones and on kainate responses in DRG neurones are also illustrated.

**Fig. 2.** Effects of LY503430 and CX-516 on native AMPA receptor activity *in vitro* and AMPA-mediated responses *in vivo*. (2a) The concentration-response profile for LY503430 (0.03-10.0 µM) potentiation was assessed by measuring the responses of acutely isolated substantia nigra dopamine neurons to 5 µM AMPA alone and in the presence of potentiator. (2b,c) Plots of the average degree of potentiation by LY503430 (0.03-10.0 µM) and CX-516 (0.3-3.0.0 mM) as a percent of the 5 µM AMPA response for each concentration of compound tested in acutely isolated substantia nigra dopamine neurons (2b) and prefrontal cortical neurons (2c). Although both compounds enhanced AMPA-evoked responses in concentration-dependent manner, LY503430 was more potent and efficacious than CX-516 in both cell types. (2d) Plot of the average change in AMPA- and NMDA-evoked discharge of CA1 hippocampal neurons in response to intravenously delivered LY503430 and CX-516. Data points in plots represent mean ± sem.

**Fig. 3.** Effects of pre-treatment for 7 days with LY503430 (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 –3.08mm caudal to bregma (B) after
MPTP treatment in mice. LY503430 provided significant protection against the MPTP-induced neurotoxicity in the substantia nigra. \( n = 6 \) per group.

**Fig. 4.** The effects of LY503430 (0.5 mg/kg s.c. for 28 days starting 1 day after infusion of 6-OHDA into the striatum) on functional outcome (A) and the number of TH immunoreactive cell bodies per slide in the substantia nigra at 5.00 mm caudal to bregma (B and C). LY503430 attenuated amphetamine (5 mg/kg i.p.) induced rotational behavior (A) the number of intact TH immunoreactive nigral cell bodies was significantly higher in LY503430-treated animals \( (p < 0.05) \). Data are based on 8 animals per group. ** \( p < 0.01 \) versus sham control, + \( p < 0.05 \) vs vehicle control.

**Fig. 5.** 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 nigra) on (A) rotational behaviour and (B and C) tyrosine hydroxylase immunoreactivity in the dorsal and ventral striatum and (D) number of TH positive nigral cell bodies. Results indicate that both doses of LY503430 provided a significant correction of apomorphine-induced rotational asymmetry (A) and loss of TH staining observed (B) in both dorsal and ventral striatum (C) after unilateral infusion of 6-OHDA into the substantia nigra. In this study the compound had minimal effects of the number of TH positive cell bodies in the substantia nigra. In parallel studies we observed some protection in the nigra after 14 days treatment with 0.5 mg/kg of LY503430. 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.
Fig. 6. The effects of chronic treatment with LY503430 (0.01 and 0.05 mg/kg p.o. for 10 days starting 1 day after infusion of 6-OHDA into the nigra) on (A and B) rotational behaviour and (C and D) tyrosine hydroxylase immunoreactivity in the dorsal and ventral striatum. Results indicate that only the 0.1 mg/kg dose of LY503430 provided a significant correction of apomorphine-induced rotational asymmetry (A) while both doses provided some protection against the loss of TH staining observed in the dorsal striatum (C and D) after unilateral infusion of 6-OHDA into the substantia nigra. Data are based on 8 animals per group. ** P < 0.01 vs baseline rotations or TH, + p < 0.05, ++ p < 0.01 vs vehicle treated animals.

Fig. 7. Dose-dependent increase in GAP-43 immunoreactivity in the striatum with LY503430 (0.05, 0.1, 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 < 0.05, ** P < 0.01 vs vehicle control.

Fig. 8. The effects of chronic treatment with LY503430 (0.05 mg/kg s.c. for 14 days starting 1 day after infusion of 6-OHDA into the nigra) on BDNF immunoreactivity in (A) the hippocampal and striatal regions of the brain and (B) the substantia nigra. Results indicated that there were similar numbers of BDNF positive cells in the hippocampal and striatal regions of sham, vehicles and LY503430-treated animals after 14 days treatment, but the LY503430-treated animals had significantly greater numbers of BDNF positive cells in the substantia nigra. Data are based on 8 animals per group. * p < 0.05 vs vehicle treated animals.

Fig. 9. Effects of delayed treatment with LY503430 (0.5 mg/kg s.c. for 14 days ) on TH immunoreactivity in the dorsal striatum after a unilateral nigral 6-OHDA lesion in
the rat. These data are based on three independent experiments and plotted together using the same axis. The data indicate that LY503430 provided similar effects on the density of TH immunoreactivity in the dorsal striatum whether administration was initiated 1, 3, 6 or 14 days after 6-OHDA. *** P < 0.01 vs sham control, + P < 0.05, ++ P < 0.01 vs vehicle control. n = 8 per group.
Table 1. Oral dose response of LY503430 on four measures of efficacy after unilateral infusion of 6-OHDA into the substantia nigra.

<table>
<thead>
<tr>
<th>Dose mg/kg</th>
<th>TH Ventral</th>
<th>TH Dorsal</th>
<th>Rotation Score</th>
<th>GAP-43</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>NS</td>
<td>p &lt; 0.05</td>
<td>NS</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>0.1</td>
<td>p = 0.052</td>
<td>p &lt; 0.05</td>
<td>p &lt; 0.01</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>0.2</td>
<td>p &lt; 0.05</td>
<td>p &lt; 0.01</td>
<td>p &lt; 0.01</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>0.5</td>
<td>p &lt; 0.01</td>
<td>p &lt; 0.01</td>
<td>p &lt; 0.01</td>
<td>p &lt; 0.01</td>
</tr>
</tbody>
</table>

The data in Table 2 were collected in three independent experiments and, to allow comparison between experiments, are tabulated in terms of significance within experiments. Each experiment has vehicle treated animals as control and data are based on eight animals per group. Results are p values calculated after ANOVA followed by appropriate post hoc t-test. NS = non-significant.

** if the data were analysed together then the minimal effective dose is 0.05 mg/kg.
EC$_{50}$ (nM) values for the effects of LY503430 on intracellular calcium flux in recombinant human GLU$_{A1-4}$ (flip and flop) receptors in expressed in HEK 293 cells

<table>
<thead>
<tr>
<th>GLU$_{A1}$ flip</th>
<th>GLU$_{A1}$ flop</th>
<th>GLU$_{A2}$ flip</th>
<th>GLU$_{A2}$ flop</th>
<th>GLU$_{A3}$ flip</th>
<th>GLU$_{A3}$ flop</th>
<th>GLU$_{A4}$ flip</th>
<th>GLU$_{A4}$ flop</th>
</tr>
</thead>
<tbody>
<tr>
<td>475 ± 102</td>
<td>4200 ± 1100</td>
<td>33.3 ± 3.31</td>
<td>2250 ± 186</td>
<td>233 ± 23.9</td>
<td>3660 ± 1140</td>
<td>98.7 ± 7.89</td>
<td>&gt;5000</td>
</tr>
</tbody>
</table>

Effects of LY503430 on kainate and NMDA receptor mediated responses

<table>
<thead>
<tr>
<th>Recombinant human transfected HEK cells</th>
<th>Native rat cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLU$_{K5}$</td>
<td>GLU$_{K6}$</td>
</tr>
<tr>
<td>&gt; 100 µM</td>
<td>&gt; 100 µM</td>
</tr>
</tbody>
</table>

Figure 1.
Figure 2.

Fold Increase from AMPA (5 μM) Response

Concentration of Potentiator (M)

LY503430
EC₅₀ = 2.7 μM

CX516
EC₅₀ > 3 mM

Fold Increase from AMPA (5 μM) Response

Percent Change from Control Firing Rate

Dose of Potentiator (μg/kg, i.v.)

LY503430 (AMPA)
\(10^{-2} \times 10^{2} \times 10^{3} \times 10^{4} \times 10^{5}\)

CX516 (AMPA)

CX516 (NMDA)

[LY503430] (μM)

0.03 0.1 0.3 1.0 3.0 10.0

LY503430
EC₅₀ = 2.8 μM

CX516
EC₅₀ = 4.8 mM

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**Figure 3.**

(a) Percentage striatal TH staining

(b) Number of TH-positive nigral cells
**Figure 4.**

(a) MEAN ASYMMETRY SCORE

(b) NUMBER OF TH POSITIVE NIGRAL CELLS

<table>
<thead>
<tr>
<th></th>
<th>SHAM</th>
<th>VEHICLE</th>
<th>LY503430</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>**</td>
</tr>
</tbody>
</table>

(c) VEHICLE TREATED

Intact | Lesioned

LY503430 TREATED

Intact | Lesioned

**Figure 4.**

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Figure 5.
Figure 6.

**Figure 6.**

The graphs illustrate the effect of different treatments on the mean asymmetry score and percentage striatal TH staining. The treatments include vehicle and LY503430 at 0.05 mg/kg p.o. and 0.1 mg/kg p.o. The data shows significant differences between baseline and apomorphine conditions, with LY503430 showing a dose-dependent effect. The p-value for the comparison between the two doses of LY503430 is 0.052.
Figure 7.

% Change in GAP-43 immunoreactivity (lesion - intact)

VEHICLE
0.05mg/kg
0.1mg/kg
0.2mg/kg
0.5mg/kg

Dose of 503430

* ** **
Figure 8.

NUMBER OF IMMUNOREACTIVE BDNF CELLS/FIELD

SHAM

VEHICLE

LY503430 (0.5mg/kg)

Cortex

CA1

Striatum

CA3

Dentate gyrus

0

5

10

15

20

25

NUMBER OF IMMUNOREACTIVE BDNF CELLS/FIELD

SHAM

VEHICLE

LY503430 (0.5mg/kg)

*
Figure 9.

PERCENTAGE STRIATAL TH STAINING

- **SHAM**
- **INTACT + LY503430**
- **VEHICLE LY503430**
- **LY503430**

Time delay before starting treatment with LY503430 (0.5 mg/kg s.c. for 14 days)

- 1 day
- 3 days
- 6 days
- 14 days

- ++
- ***
- +

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