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Evaluation of the mGluR2/3 agonist LY379268 in rodent models of Parkinson's disease

Tracey K. Murray[a], Marcus J. Messenger[b], Mark A. Ward[a], Sandra Woodhouse[a], David J. Osborne[a], Susan Duty[b], Michael J. O'Neill[a,*]

[a]*Lilly Research Centre, Eli Lilly and Co. Ltd., Erl Wood Manor, Windlesham, Surrey GU20 6PH, UK*
[b]*Neurodegenerative Disease Research Group, Wolfson Centre for Age Related Diseases, Hodgkin Building, GKT School of Biomedical Sciences, King's College London, London SE1 1UL, UK*

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Abstract

The aim of the present studies was to examine the ability of a potent, systemically active, selective Group II mGlu receptor (mGluR2/3) agonist, 1*R*,4*R*,5*S*,6*R*-2-oxa-4-minobicyclo[3.1.0.]hexane-4,6-dicarboxylate (LY379268) to provide both functional relief and neuroprotection in rodent models of Parkinson's disease (PD). In functional studies, intracerebroventricular administration of LY379268 (1, 5, 10, 20 nmol/2 μl) produced a dose-dependent increase in locomotor activity in the reserpine (5 mg/kg ip)-treated rat. In contrast, systemic administration of LY379268 (0.1, 1, 10 mg/kg ip) did not reverse reserpine-induced akinesia and failed to effect rotational behaviour 1 month after unilateral lesioning of the nigrostriatal tract by 6-hydroxydopamine (6-OHDA; 4 μg infused into the substantia nigra (SN)). In neuroprotective studies, animals were treated with LY379268 (10 mg/kg/day ip) either for 7 days following 6-OHDA injection into the SN (4 μg) or for 21 days following 6-OHDA injection into the striatum (10 μg) before measurement of tyrosine hydroxylase immunoreactivity in the striatum and/or SN as an index of neuroprotection. LY379268 provided some protection against nigral infusion of 6-OHDA and also some functional improvement and correction of dopamine turnover was observed. The compound also provided significant protection in the striatum and some protection in the SN against striatal infusion of 6-OHDA. These data suggest that activation of Group II mGlu receptors can provide some protection in models of PD, while their role in providing functional improvement is less clear.

Keywords: Akinesia; Glutamate; Metabotropic glutamate receptor; LY379268; Parkinson's disease; Neuroprotection; Reserpine; 6-Hydroxydopamine

1. Introduction

Parkinson's disease (PD) is a chronic movement disorder resulting from a disturbance in the normal functioning of the basal ganglia, a collection of subcortical nuclei that are essential for the initiation and control of motor activity. The underlying pathology of the disease is a progressive degeneration of the dopaminergic nigrostriatal tract that manifests as a range of motor deficits including akinesia or bradykinesia, tremor, rigidity and postural instability. Current therapies for PD are essentially based on dopamine replacement and include levodapa (L-DOPA), a precursor of dopamine, and dopamine receptor agonists. These agents are effective in treating the symptoms of the disease in the early stages, but are less effective as the disease progresses when debilitating side-effects such as "on–off" fluctuations in efficacy and uncontrollable dyskinesias ensue. More importantly, dopaminergic treatments do not halt the disease progression. For these reasons, several investigators have started to focus on nondopaminergic interventions as symptomatic and neuroprotecive strategies in PD.

It is well established that the loss of striatal dopaminergic innervation in PD produces downstream changes in the basal ganglia circuitry that include increased activity of the glutamatergic subthalamic nucleus (STN) (Mitchell et al., 1989). This overactivity can lead to increased glutamate-mediated excitation in the basal ganglia output regions, substantia nigra pars reticulata (SNr) and globus pallidus internus (GPi), which may in turn lead to reduced thalamocortical feedback and the subsequent appearance of akinesia. Tar-

* Corresponding author. Tel.: +44-1276-483547; fax: +44-1276-483525.
E-mail address: oneill_michael_j@lilly.com (M.J. O'Neill).

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geting this excess glutamatergic transmission may, therefore, be one useful nondopaminergic approach in the symptomatic treatment of PD. This idea is backed up by a number of observations including the success of surgical interventions such as subthalamotomy in alleviating akinesia in PD patients (Bergman et al., 1990) and studies showing that direct infusion of ionotropic glutamate antagonists into the GPi or SNr can alleviate symptoms in animal models (Starr et al., 1997). Recent studies have focused on the role of metabotropic glutamate (mGlu) receptors in these pathways. Group I mGlu receptors (mGlu1 and 5) are coupled to phosphoinositide (PI) hydrolysis and are predominantly located postsynaptically (Shigemoto et al., 1993). In contrast, Groups II (mGlu2 and 3) and III (mGlu4, 6, 7 and 8) mGlu receptors are negatively coupled to adenylyl cyclase and are thought to act as presynaptic autoreceptors, regulating glutamate transmission (Shigemoto et al., 1997). Activation of Group II or III mGlu receptors may, therefore, offer a way of reducing the excess glutamatergic drive of the SNr and GPi, thereby relieving PD symptoms.

Studies in this laboratory have previously demonstrated that intranigral or intracerebroventricular injection of the Group II agonist ((2*S*,1′*R*,2′*R*,3′*R*)-2-(2,3-dicarboxycyclo propyl)glycine) DCG-IV alleviates akinesia in the reserpine-treated rat model of PD (Dawson et al., 2000) by a mechanism that likely involves inhibition of glutamate release from STN efferents in the SNr. Many of the earlier Group II mGlu receptor agonists, including DCG-IV, were not, however, ideal tools since they exhibited cross-reactivity with other mGlu and iGlu receptor subtypes and did not cross the blood brain barrier, reducing their compliance for many in vivo studies. The discovery at Eli Lilly and Co. of a series of potent and selective mGluR2/3 agonists with systemic activity, of which (+)-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid (LY354740) was the parent compound (Monn et al., 1997; Schoepp et al., 1997), has opened up new possibilities. LY354740 (5 and 10 mg/kg ip) has already been shown to dose-dependently block haloperidol-induced muscle rigidly in rats (Konieczny et al., 1998). In addition, elegant electrophysiological studies have shown that LY354740 inhibits synaptic excitation of the SNr (Bradley et al., 2000) and that this inhibition is blocked by the selective Group II antagonist, 2*S*-2-amino-2-(1*S*,2*S*-2-caroxycyclopropy-1-yl)-30(xanth-9-yl)propionic acid (LY341495).

By virtue of their autoreceptor role, the possibility that Group II mGlu receptor agonists may offer protection against glutamate-mediated neurodegeneration has also been explored (for review, see Nicoletti et al., 1996; O'Neill, 2001). Early studies have the demonstrated neuroprotective effects in vivo with mGluR agonists. For example, DCG-IV blocks kainic acid-induced degeneration in rats (Miyamoto et al., 1997) and *trans*-amino-cyclopentane dicarboxylic acid (*trans*-ACPD) reduces infarct size in a mouse model of focal ischaemia (Chiamulera et al., 1996). More recently, LY354740 and the more potent analogue LY379268 have been shown to provide neuroprotection against NMDA-mediated cell death in rat cortical neuronal cultures (Kingston et al., 1999). LY379268 has also been shown to provide almost complete protection against CA1 hippocampal cell damage in vivo in the gerbil model of cerebral ischaemia, in a LY341495-sensitive manner (Bond et al., 2000). It has been suggested that the protection may be due to activation of mGuR3 and an increase in growth factor secretion (Bruno et al., 1998). Recent studies have reported that DCG-IV protects against MPP^+-induced neurotoxicity and increases BDNF mRNA expression in the striatum (Matarredona et al., 2001).

In summary, our laboratories have previously demonstrated that (1) central administration of DCG-IV-alleviated reserpine-induced akinesia (Dawson et al., 2000) and (2) that Group II mGlu receptor agonists could provide in vitro (Kingston et al., 1999) and in vivo protection against glutamate toxicity (Bond et al., 2000). The aims of the present studies were to examine the functional effects of the potent and systemically active Group II agonist LY379268 in reserpine-treated and 6-OHDA-lesioned rats, and to evaluate the neuroprotective effects of LY379268 after infusion of 6-OHDA into the SN or striatum. A combination of behavioural, neurochemical and histological techniques were employed.

2. Methods

2.1. Animals

Male Sprague–Dawley rats (Harlan, UK or Tuck and Son, UK) weighing 280–320 g were used. Animals were housed in groups of 5–6, in a temperature-and humidity-controlled environment with a 12-h light/12-h dark cycle, and food and water available ad libitum.

2.2. Drugs and chemicals

6-Hydroxydopamine (6-OHDA) hydrobromide, desipramine HCl, pargyline HCl, reserpine and β-cyclodextrin were all obtained from Sigma-Aldrich (Dorset, UK). LY379268 was synthesised on site at Eli Lilly and Co., Indianapolis, USA.

2.3. Experimental models of PD

All experiments were carried out in accordance with the Home Office Animals (Scientific Procedures) Act, UK, 1986.

2.3.1. 6-OHDA-induced lesions of the nigrostriatal tract

For all lesion models, rats were anaesthetised with a gaseous anaesthetic consisting of Isoflurane/nitrous oxide/oxygen (induction: 3 l/min O_2, 5% Isoflurane; maintenance: 3 l/min N_2O, 1.5 l/min O_2, 1.5–5% Isoflurane, adjusted according to each rat's respiration rate and reflex response). Once anaesthetised, rats were placed on a thermostatically

controlled heating blanket to maintain body temperature within the range 37–38 °C. They were then placed in a Kopf stereotaxic frame and the scalp incised so as to uncover the parietal bones.

2.3.1.1. 6-OHDA infusion into the substantia nigra (SN). Unilateral lesions of the nigrostriatal tract were induced by stereotaxic infusion of 6-OHDA (4 μg free base in 1.8 μl 0.02% ascorbic acid in 0.9% saline) into the left SN at the following coordinates (according to Paxinos and Watson, 1986): AP: −4.8 mm, L: +1.9 mm, relative to the bregma, V: −8.0 mm (from skull surface) and incisor bar −3.7 mm below the interaural line. 6-OHDA was infused using a Sp100i single syringe infusion pump with a 25-μl 1000 series Hamilton syringe connected to a 28-gauge steel cannula. The infusion was made over a period of 6 min at a rate of 0.3 μl/min, followed by 2-min equilibration time when the needle remained in place. Sham-operated rats received identical surgery to the 6-OHDA group but 1.8 μl 0.02% ascorbic acid (6-OHDA vehicle) was infused. Thirty minutes prior to 6-OHDA infusion rats were pretreated with desipramine (12.5 mg/kg ip) to block uptake of the toxin into noradrenergic neurons and pargyline (75 mg/kg ip) to block metabolism of the toxin.

2.3.1.2. 6-OHDA infusion into the striatum. Unilateral lesions of the nigrostriatal tract were induced by stereotaxic infusion of 6-OHDA (10 μg free base in 2.6 μl 0.02% ascorbic acid in 0.9% saline) into the right striatum at the following coordinates (according to Paxinos and Watson, 1986): AP: 0.7 mm, L: −2.3 mm relative to bregma, V: −6.0 mm (from skull surface) and incisor bar −3.7 mm below the interaural line. 6-OHDA was infused using a Sp100i single syringe infusion pump with a 25-μl 1000 series Hamilton syringe connected to a 28-gauge steel cannula. The infusion was made over a period of 4 min at a rate of 0.643 μl/min, followed by 4 min equilibration time, with the needle remaining in place. Sham-operated rats received identical surgery to the 6-OHDA group, but 2.6 μl 0.02% ascorbic acid (6-OHDA vehicle) was infused. Thirty minutes prior to 6-OHDA infusion, rats were pretreated with desipramine (12.5 mg/kg ip) and pargyline (75 mg/kg ip).

2.3.1.3. Postoperative care. On completion of the stereotaxic infusion of 6-OHDA the incision was sutured and rats were administered 5 ml of saline to aid recovery from the surgical procedure. The animals were placed in temperature-regulated incubators (thermacages) for 3–6 h and then returned to their home cages. Animals were visually checked at regular intervals and weighed daily until the end of the experiment.

2.3.2. Intracerebroventricular cannulation and induction of reserpine-induced akinesia

Under general anaesthesia (halothane; 4% induction to 2% maintenance in 95% O_2/5% CO_2 at 2 l/min), some animals were stereotaxically implanted, with 23-gauge stainless steel guide cannulae, 2 mm above the third ventricle (AP: 4.3 mm, L: 0 mm and V: −3.7 mm from skull surface; coordinates relative to bregma according to Paxinos and Watson, 1986). Cannulae were maintained patent by insertion of a temporary stainless steel 30-gauge stylet. Following a minimum 4-day recovery period, cannulated and naive rats were treated with reserpine (5 mg/kg sc) to induce catecholamine depletion and subsequent akinesia. Eighteen hours later, when animals displayed a stable level of akinesia, the effects of the Group II mGlu receptor agonist, LY379268, were assessed on motor behaviours as detailed below.

2.4. Evaluation of functional effects of LY379268

2.4.1. In rats bearing a unilateral lesion of the nigrostriatal tract

Unilateral lesions of the nigrostriatal tract were induced by 6-OHDA injection into the SN (as described in Section 2.3.1.2) 1 month prior to testing. Animals were placed in automated rotometers (Med. Associates). The apparatus consisted of perspex bowls where each rat was linked to a harness that had an infrared sensor at the top. The sensor detected clockwise and anticlockwise movement, and was coupled to a computer with ROTORAT software, which was capable of measuring full and partial rotations. The number of contraversive and ipsiversive rotations was measured over a given time and this was then expressed as a rotational asymmetry score (contraversive minus ipsiversive rotations). The animals were tested for baseline rotations and then challenged with amphetamine (5 mg/kg ip) to confirm the success of lesions by observation of a marked negative rotational asymmetry score. The animals were then left for 2 weeks and after this time each animal then received injections of LY379268 (1, 2.5, 5 and 10 mg/kg with 7-day intervals between each dose to allow wash-out) and locomotor assessments were made, as described above, on each occasion.

2.4.2. In reserpine-treated rats

For all studies, visual locomotor assessments were performed in rectangular cages with 5 cm^2 grid lines covering the base. Following an initial 15-min period of acclimatisation, baseline akinetic activity was videotaped for 30 min. Some animals ($n=4–5$ per dose) then received a single systemic injection of LY379268 (0.1, 1 or 10 mg/kg ip) or saline. Those previously cannulated received a single intraventricular injection of LY379268 (1, 5, 10 and 20 nmol in 2 μl PBS, pH 7.4) or vehicle (2 μl PBS) over a 2-min period ($n=5–6$ rats per group). Animals were videotaped for a further 60 min. Locomotor activity was measured manually, by observation of videotape recordings. Activity was quantified in 5-min time-bins over a maximum 60-min period in arbitrary locomotor units (ALUs), where 1 ALU refers to both front paws crossing a grid line. Mean data are represented in 5-min time bins or as the sum of ALUs over a

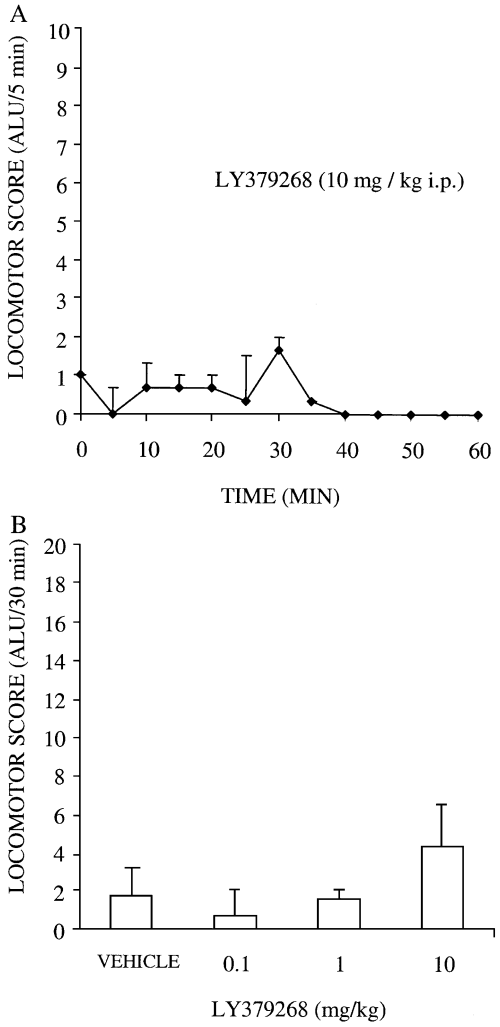

Fig. 1. Locomotor effects following systemic administration of LY379268 in the reserpine-treated rat. (A) Time course of locomotor activity induced by a 10-mg/kg dose of LY379268 and (B) lack of dose-related locomotor effects of LY379268 (0.1–10 mg/kg ip). Values represent mean ± S.E.M. ($n = 4–5$ animals per dose).

30-min period. In some animals ($n = 4$), two identical doses of LY379268 (5 nmol/2 μl icv) were given at 24-h intervals, in order to examine the stability of locomotor responses observed.

2.5. Experimental protocols for neuroprotective studies

Three studies were carried out to evaluate the neuroprotective ability of LY379268, the first two using an almost total lesion of the nigrostriatal tract, the second using a more partial lesion model. In the first, LY379268 (10 mg/kg ip) was administered chronically, 1 h before and again at 1, 2, 3, 4, 5, 6 and 7 days after unilateral nigral infusion of 6-OHDA (4 μg) and the brains removed for assessment of TH immunoreactivity on Day 10. In the second, Study 1 was repeated, but this time the left and right striata were removed at Day 10 for subsequent measurement of dopamine and its metabolites. In both nigral studies, we also measured baseline and apomorphine (0.25 mg/kg)-stimulated rotational behaviour (as outlined in Section 2.4.1 above) prior to killing ($n = 8$ per group).

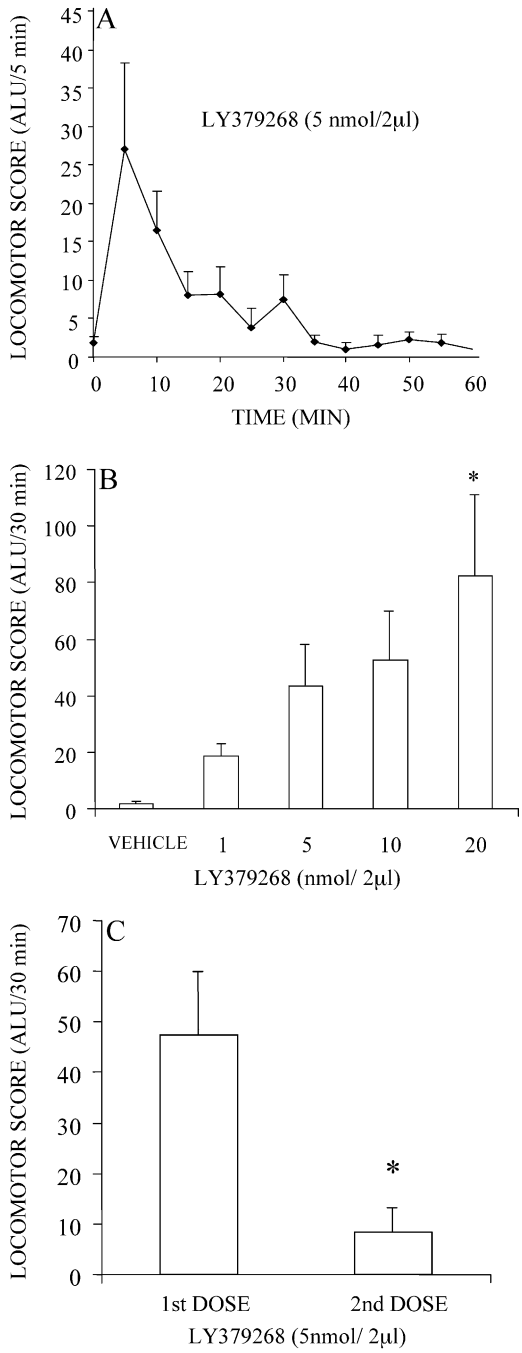

Fig. 2. Locomotor effects following intracerebroventricular administration of LY379268 in the reserpine-treated rat. (A) Time course of locomotor response following intracerebroventricular administration of LY379268 (5 nmol in 2 μl). (B) Dose-related increase in locomotor activity following intracerebroventricular administration of LY379268 (1–20 nmol in 2 μl) or vehicle (2 μl PBS). (C) Diminished locomotor response to LY379268 (5 nmol in 2 μl) on repeated administration (24 h apart). Values represent mean ± S.E.M. ($n = 4–7$ animals per dose). * $P < .05$ vs. vehicle effect or first dose of LY379268.

For the partial lesion model, we had previously demonstrated that infusion of 10 μg of 6-OHDA into the striatum produces a slow partial (40–50%) lesion that reaches maximum at 4 weeks after infusion of 6-OHDA (Murray et al., in press). Therefore, in the final study, LY379268 (10 mg/kg ip) was administered daily for 21 days starting 6 h after infusion of 6-OHDA into the striatum and the brains were removed for TH immunostaining and nigral cell analyses on Day 28.

2.6. Evaluation of neuronal damage

2.6.1. General histology

For all studies, at the experiment endpoints, the rats were given an overdose of anaesthetic, the thorax opened and perfused with 30 ml of saline followed by 30 ml of 10% buffered formalin via the left ventricle or vena cava. The brains were removed and placed in vials containing 10% buffered formalin for 1–3 days. The brains were cut into 2×6 mm segments using a rodent brain matrix, and the segments processed and embedded in paraffin wax. Processing was carried out using an automated machine (TISSUE-TEK VIP 2000, Vacuum Infiltration Processor from Miles Scientific, Bayer Diagnostics). Once the segments were embedded, 8-μm coronal sections were taken using a microtome (Leitz 1400 sledge microtome). The striatum was sectioned at the level 1.2 mm rostral to bregma. Several sections were taken through the SN at the level 4.8–5.3 mm caudal to bregma.

2.6.2. Tyrosine hydroxylase immunostaining (TH-I)

TH-I was performed at both striatal and SN levels. Sections were deparaffinised and rehydrated. Nonspecific binding was blocked with 1.5 % normal goat serum (Vec-

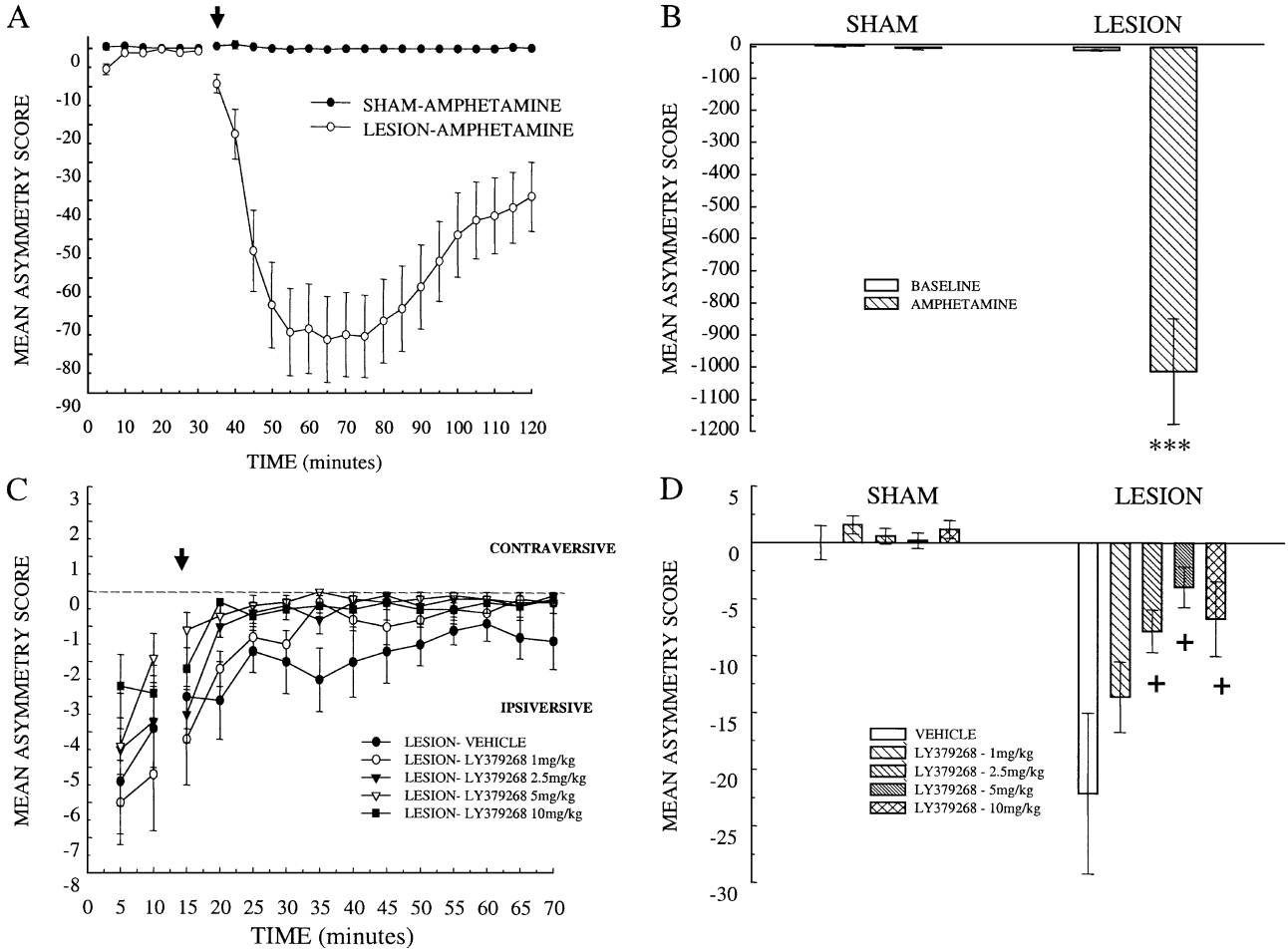

Fig. 3. The effect of (top panels) vehicle or amphetamine (5 mg/kg ip) on (A) rotational behaviour over 120 min and (B) total counts for 90 min in sham- and 6-OHDA-lesioned animals. The effect of (bottom panels) LY379268 (1–10 mg/kg ip) on (C) rotational behaviour over 70 min in 6-OHDA-lesioned rats and (D) total counts for 60 min in sham- and 6-OHDA-lesioned rats. Arrows indicate the time of administration of amphetamine and LY379268 in (A) and (C), respectively. The results indicate that amphetamine has no effect in sham-operated animals, but produces a large negative change in asymmetry scores in lesioned animals. LY379268 failed to alter rotational behaviour in sham-operated animals, but, in contrast to amphetamine, systemic administration of LY379268 produced negligible changes in rotational behaviour in lesioned animals. In fact, LY379268 reduced the residual negative asymmetry score present in vehicle lesioned animals. All data are expressed as mean asymmetry scores (defined in Section 2) ± S.E.M. ($n = 8$ animals per group). ***$P < .001$, amphetamine vs. vehicle; $^{+}P < .05$, LY379268 vs. vehicle.

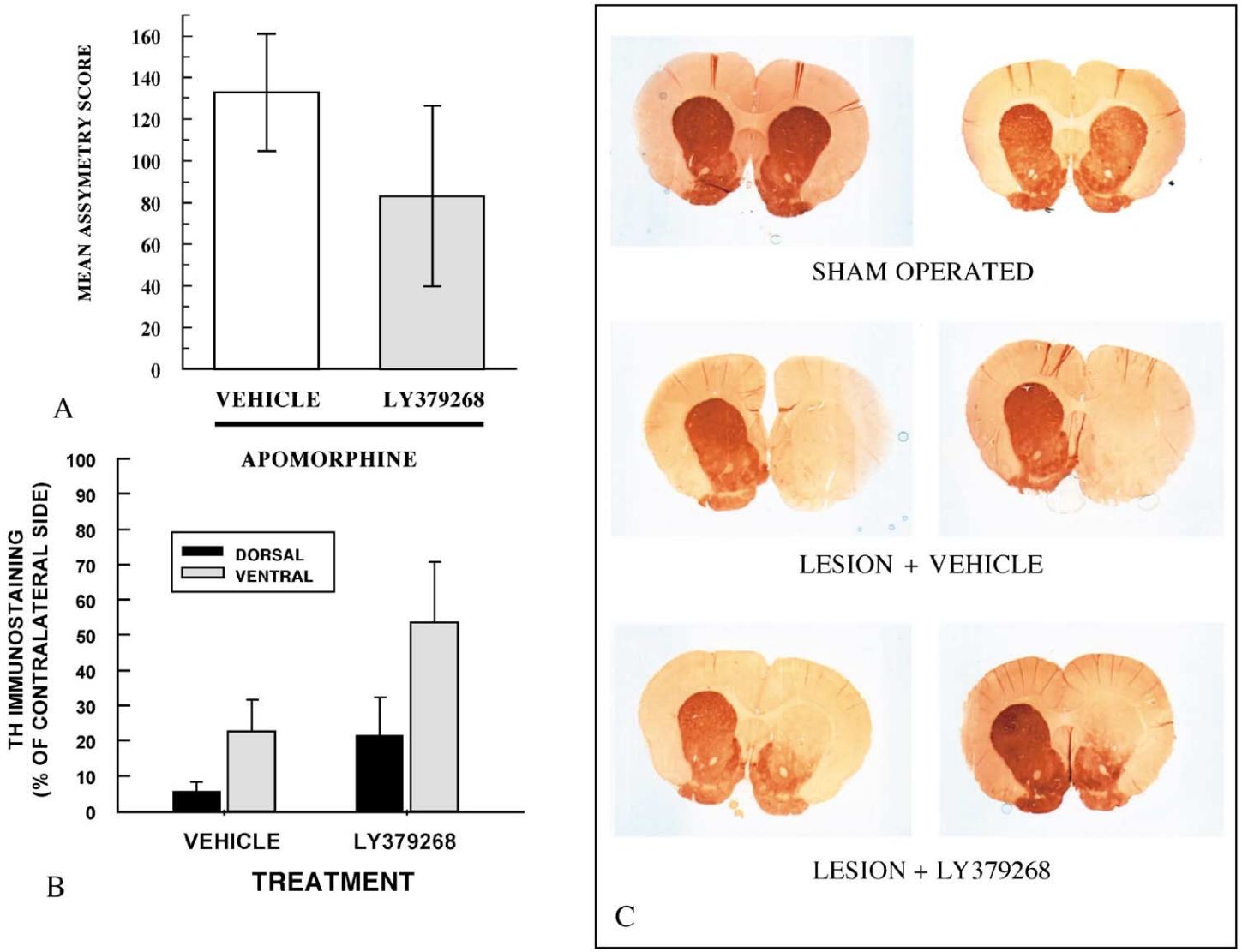

Fig. 4. The effects of chronic treatment with LY379268 (10 mg/kg for 7 days starting 6 h after infusion of 6-OHDA into the nigra) on (A) rotational behaviour and (B, C) tyrosine hydroxylase immunoreactivity in the dorsal and ventral striatum. Results indicate that LY379268 provided some correction of apomorphine-induced rotational asymmetry and loss of TH staining seen after unilateral infusion of 6-OHDA into the SN. Data are based on eight animals per group.

tastain rabbit IgG ABC kit). This was followed by application of rabbit polyclonal anti-TH antibody (diluted 1:100 in PBS, Chemicon AB152) incubated for 18 h at room temperature. After washing in PBS, the sections were incubated with the biotinylated secondary antibody (Vectastain rabbit IgG ABC kit) for 30 min after which they were washed again and the HRP conjugate (Vectastain rabbit IgG ABC kit) was applied for 30 min followed by PBS rinses. Finally, they were visualised with diaminobenzidine. The colour was developed for 6–10 min and the staining terminated by washing with tap water. The stained sections were then dehydrated, cleared and cover slipped.

For the striatum, the extent of dopamine terminal cell loss was measured by density analysis using an image analysis system (Optimas 5.2). TH-I density for both dorsal and ventral striatum of each hemisphere was measured. TH-I for the lesioned hemisphere was then expressed as a percentage of TH-I for the respective intact hemisphere.

The nigral sections were examined microscopically. In a representative slide, the number of TH positive cell bodies was counted. Each cell was also assigned a morphology score from 1 to 4 (4 indicates an intact, round cell with clear nucleus and cytoplasm, whereas the lower values progressively indicate shrinkage, irregular forms and loss of cytoplasm and nucleus) and a dendrite score from 1 to 4 (4 indicates a large number of intact densities, whereas the lower scores progressively indicate loss of dendrites).

2.6.3. Measurement of dopamine and metabolites

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

2.7. Statistical analysis

In 6-OHDA-lesioned rats, statistical analysis of data was carried out using analysis of variance (ANOVA) followed by post-hoc Dunnett's test to compare drug treatment groups with the vehicle. Statistical analysis of histological and biochemical data was assessed using ANOVA followed by Student's *t*-test. In reserpine-treated rats, different doses of LY379268 were compared using a one-way analysis of variance and Student's *t*-test, while first and second doses were compared using a paired *t*-test. In all cases, $P < .05$ was considered to be statistically significant.

3. Results

3.1. Functional effects of LY379268

3.1.1. Effects on locomotor activity in reserpine-treated rats

During the baseline periods, all the reserpine-treated rats exhibited negligible locomotor activity (between 0 and 6 in arbitrary locomotor units 30 min^{-1}) and were thus considered suitably akinetic for inclusion in the study. Results indicated that systemic administration of LY379268 (0.1, 1 or 10 mg/kg ip) failed to induce locomotor activity in reserpine-treated animals (Fig. 1).

In contrast, following intracerebroventricular infusion of LY379268 locomotor activity in reserpine-treated rats, an increase in locomotor activity was observed. This peaked within 5 min of injection and had subsided by 30 min, as shown for a single 5-nmol dose (Fig. 2A). Quantification of total locomotor activity over a 30-min period indicated that LY379268 (1, 5, 10 and 20 nmol) produced a dose-dependent increase in locomotor activity that reached statistical significance at the highest dose tested (Fig. 2B). However, the effects of LY379268 were much diminished on second exposure to the drug. Thus, the mean second response to 5-nmol LY379268 was reduced by more than 80% compared to the initial response recorded 24 h earlier ($P < .05$) (Fig. 2C).

3.1.2. Effects on rotational behaviour in rats bearing a nigrostriatal tract lesion

The rotational data obtained are shown in Fig 3 where A and C show the time-course of the behavioural responses,

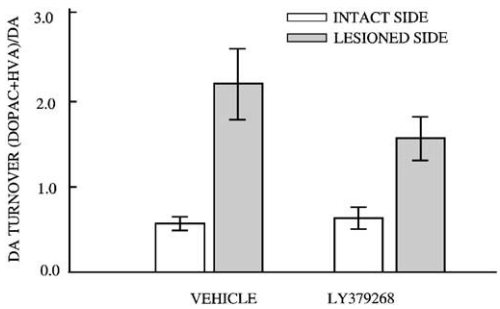

Fig. 5. The effects of LY379268 (10 mg/kg for 7 days) on striatal dopamine turnover (DOPAC + HVA/DA) in the lesioned and intact hemispheres 14 days after infusion of 6-OHDA in the SN. Results indicate that the predicted 6-OHDA-induced increase in striatal dopamine turnover in the lesioned side is partially attenuated by LY379268. Data are mean ± S.E.M. ($n = 8$).

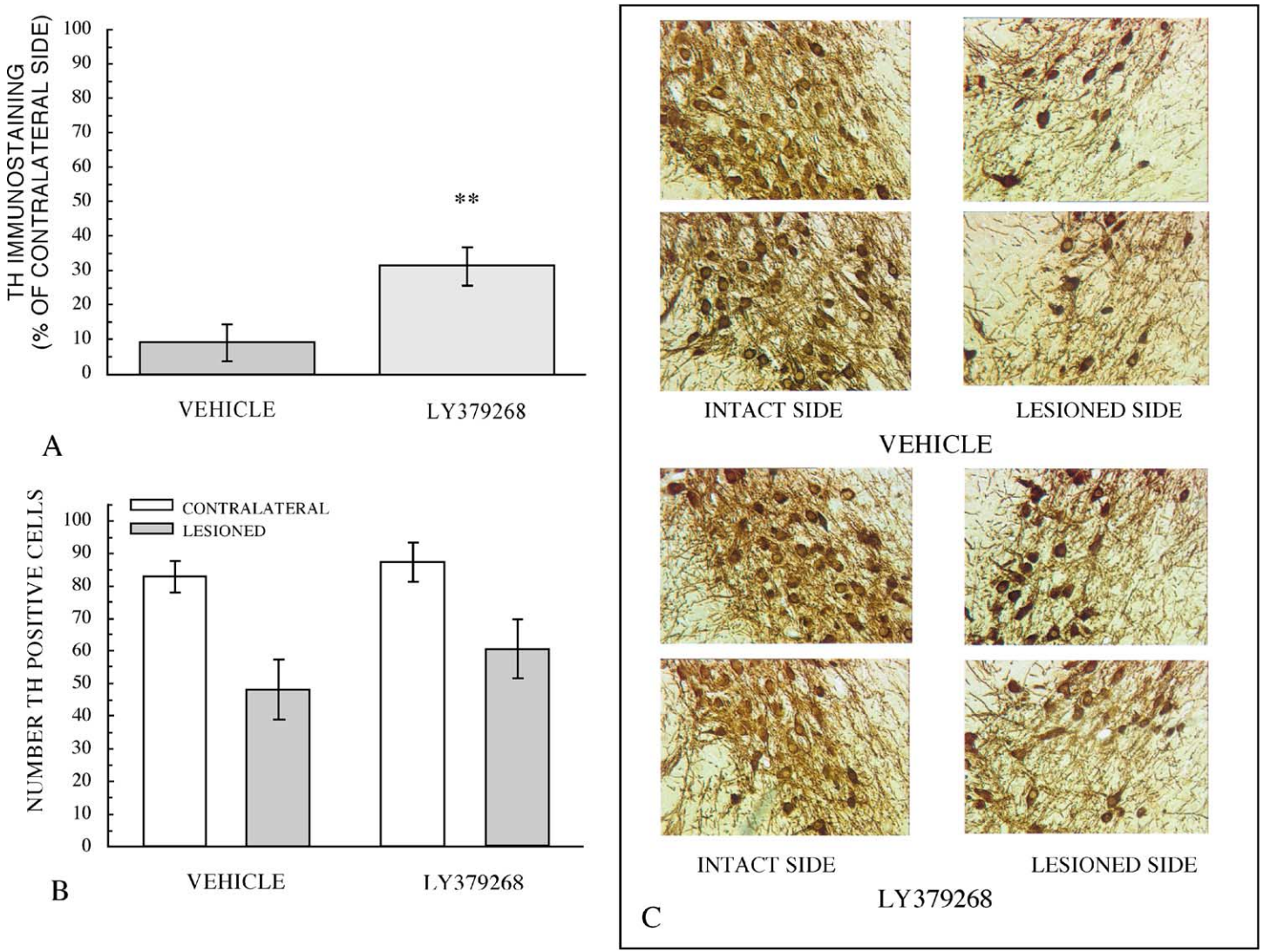

Fig. 6. The effects of LY379268 (10 mg/kg for 21 days starting 1 day after infusion of 6-OHDA into the striatum) on TH immunoreactivity in the striatum (A) and SN (B, C). Remaining TH-I in the striatum was significantly higher in LY379268-treated animals compared to vehicle-treated animals, indicating significant protection ($P < .01$). LY379268 also provided some protection in the SN, but this failed to reach significance. Data are based on eight animals per group. $^{**}P < .01$ vs. vehicle control.

while B and D show the total asymmetry score from the time of injection until the end of the rotational experiments (over 60- or 90-min period). One-month post 6-OHDA lesion, injection of amphetamine (5 mg/kg ip) produced a marked typical negative change in asymmetry scores (reflecting a bias towards ipsiversive rotations), thus, confirming that the nigrostriatal tract had been successfully lesioned. This effect was most pronounced 30 min after amphetamine, but was still present 60 min after injection (Fig. 3A and B). Sham-lesioned animals produced no such asymmetry in locomotor activity with amphetamine injection. In these same groups, LY379268 (1, 2.5, 5 and 10 mg/kg ip) had minimal effect on rotational behaviour (Fig. 3C and D). The vehicle-treated 6-OHDA-lesioned animals had a negative asymmetry score (indicating basal net ipsiversive rotations), though this was of a negligible level when compared to that of amphetamine. At the higher doses tested, LY379268 appeared to decrease the level of this, mainly because the compound appeared to make the animals less active. This was confirmed by using the software to measure the total movement made in the rotometer. Results indicated that total movement decreased for both sham and lesioned animals at the 2.5, 5 and 10 mg/kg doses (data not shown). However, the LY379268-treated animals still responded to disturbances indicating that sedation was not a factor.

3.2. Neuroprotective effects of LY379268

3.2.1. Effects on rotational behaviour, TH immunoreactivity and striatal dopamine and metabolites in rats bearing a nigrostriatal tract lesion induced in the SN

Animals were treated daily for 7 days with vehicle or LY379268 (10 mg/kg ip) commencing 6 h after 6-OHDA lesion. Results indicated that infusion of 6-OHDA into the SN produced a large increase in apomorphine (0.25 mg/kg sc)-induced rotations. Treatment with LY379268 produced a slight attenuation of the apomorphine-induced rotations, but this failed to reach significance (Fig. 4A). Results of TH immunocytochemistry indicated that there was a large loss in TH staining in the striatum of vehicle-treated animals (Fig. 4C). Quantification of the staining indicated that this amounted to a 95% loss of TH staining in dorsal and 75% loss in ventral striatum. Treatment with LY379268 provided some protection (loss of TH staining was 80% in dorsal and 45% in ventral striatum; Fig. 4B), although this protection failed to reach statistical significance (P=.09 and .06, respectively).

In a second series of animals, treated as above, tissue dopamine, DOPAC and HVA levels were measured in the left and right striata. As expected, striatal dopamine turnover (DOPAC + HVA/DA) was increased in the lesioned striatum following 6-OHDA infusion in the SN (Fig. 5). Treatment with LY379268 provided some attenuation in the 6-OHDA-induced alterations in dopamine turnover. However, this failed to reach statistical significance.

3.2.2. Effects on rotational behaviour and TH immunoreactivity in rats bearing a nigrostriatal tract lesion induced in the striatum

In vehicle-treated animals bearing a partial unilateral lesion of the nigrostriatal tract, TH-I was reduced by approximately 90% in the striatum in the lesioned side compared to the intact side (which was set as 100%, data not shown) (Fig. 6A). The number of TH positive cells in the SN fell by some 55% in the lesioned side compared to the intact side (Fig. 6B). Chronic treatment with LY379268 (10 mg/kg ip for 21 days) provided significant protection in the striatum (Fig. 6A) and also provided some protection of the nigral cell bodies, but this failed to reach statistical significance (Fig. 6B,C).

4. Discussion

These studies evaluated the functional and neuroprotective effects of LY379268, a potent and systemically active Group II mGlu receptor agonist, in rodent models of PD. The data indicate that, while systemic administration of LY379268 failed to reverse reserpine-induced akinesia or to produce typical anti-parkinsonian rotational behaviour in animals bearing a near complete unilateral lesion of the nigrostriatal tract, central (intracerebroventricular) administration did provide some alleviation of the reserpine-induced akinesia. In addition, the data reveal that LY379268 provides some degree of neuroprotection in animals bearing a near complete or partial lesion of the nigrostriatal tract.

Increased glutamatergic excitation of the basal ganglia output regions (GPi and SNr), resulting from overactivity of the STN, is thought to contribute to the reduction in thalamocortical feedback (Mitchell et al., 1989) and thus the akinetic symptoms of PD. Overactivity of the STN may also cause increased glutamatergic innervation of the SNc and this has been suggested to contribute to excitotoxic degeneration of SNc neurones in PD (Rodriguez et al., 1998). Subthalamotomy has proven a useful surgical approach in the treatment of PD patients (Bergman et al., 1990). However, a pharmacological means of achieving this effect would be more desirable to patients and neurologists alike. Since Group II mGlu receptors present on STN efferent terminals are known to act as autoreceptors, regulating the release of glutamate (e.g., Nicoletti et al., 1996), agonists selective for these receptors have been suggested to provide a suitable potential pharmacological target. With this in mind, the present studies were designed to examine the hypothesis that Group II mGlu receptor agonists may provide an alternative nondopaminergic treatment for PD.

In the present study, the reversal of reserpine-induced akinesia seen following intracerebroventricular administration of LY379268 suggests a role for Group II mGlu receptor activation in alleviating akinesia in PD. This finding is also consistent with previous reports from these laboratories showing that injection directly into the SNr or

intracerebroventricular administration of DCG-IV could alleviate akinesia in reserpine-treated rats (Dawson et al., 2000). That a second intracerebroventricular administration of LY379268 had a much smaller anti-akinetic effect suggests that the cellular mechanism behind this response is subject to some desensitisation. This apparent desensitisation was not observed with DCG-IV in the previous studies (Dawson et al., 2000) and the reason behind these differences is not known.

These earlier studies with DCG-IV, which does not penetrate the blood brain barrier, were not able to assess the effectiveness of systemic application of a Group II mGlu receptor agonist in reversing akinesia. However, with the recent identification of a family of potent, selective and systemically active Group II mGlu receptor agonists including LY354740 and LY379268 (Schoepp et al., 1997; Monn et al., 1999; Kingston et al., 1999; Schoepp et al., 1999), testing this route of administration is now feasible. This family of systemically active molecules has already been shown to depress excitation (EPSC generation) in the SNr (Bradley et al., 2000) via a presynaptic mechanism that is blocked by the Group II mGlu receptor antagonist, LY341495. This paper also reported that systemic administration of LY354740 reversed haloperidol-induced catalepsy in rats, consistent with previous reports that LY354740 could attenuate haloperidol-induced muscle rigidity in rats (Konieczny et al., 1998).

In the present study, despite the success of intracerebroventricular administration of LY379268, systemic administration of this agonist failed to reverse reserpine-induced akinesia. However, the reserpine model is severe, and indeed high does of other interventions such as ionotropic glutamate antagonists are required for efficacy in this model. In addition, in rats bearing a near complete lesion of the nigrostriatal tract, while LY379268 produced a small reduction in locomotor activity, no increase in net contraversive rotations was observed, reflecting a further lack of anti-parkinsonian behavioural response in this rodent model of PD.

One obvious explanation for the lack of functional effect following systemic administration of LY379268 could be that the compound fails to penetrate the brain or does not reach sufficient levels. However, we have previously demonstrated that systemic administration of LY379268 penetrates the brain rapidly and is active in animal models of global ischaemia following systemic administration (Bond et al., 2000). Other studies have also reported that LY379268 is effective in a variety of experimental situations following systemic administration, for example, in reducing PCP- (Cartmell et al., 2000a) or amphetamine (Cartmell et al., 2000b)-induced behaviours and in reducing seizures in rodent models of epilepsy (Moldrich et al., 2001).

More relevant to the current studies are recent reports that demonstrate that LY379268 increases dopamine and serotonin turnover in the rat prefrontal cortex, nucleus accumbens and striatum (Cartmell et al., 2000c). These authors subsequently demonstrated, using in vivo microdialysis, that systemic administration of LY379268 increased monoamine release and that this could not be produced by local infusion (Cartmell et al., 2001). These results suggest that activation of Group II mGlu receptors can produce alterations in release and tissue levels of monoamines simultaneously in various neuronal circuits in the rat brain. Therefore, one likely explanation for the lack of systemic activity in the current studies is due to complex interactions in the SNr (intended site of action), thalamus, cortex and striatum that cancel one another out. This may be particular likely to occur when dealing with a complex neuronal circuit like the thalamocortical feedback loop that controls motor behaviours. Taking all this data together is possible that LY379268 may produce opposite effects in different parts of the brain and these opposing effects interact so as we fail to alter movement after systemic administration. However, that said, it is still curious why intracerebroventricular administration of LY379268 should remain effective, considering that Group II mGlu receptors in more than one brain region may also be expected to be activated via this route of administration. It is also clear that Group II mGluRs are located pre- and postsynaptically. Thus, while presynaptic actions may well decrease glutamate release, there may also be additional effects from activation of postsynaptic receptors that may contribute to the observed lack of efficacy.

The second aspect of the current studies was to evaluate the neuroprotective effects of LY379268 in rats bearing near complete and partial 6-OHDA-induced lesions of the nigrostriatal tract. The data indicate that chronic administration of LY379268 provides some protection in both models. The level of protection following 6-OHDA infusion directly into the SN is not large, but the model is very severe (90% loss of TH staining in dorsal striatum) and the effects observed are similar, or better than, other interventions that have been reported to protect in PD models. Thus, the effects of LY379268 are similar to our results with nicotinic receptor agonists (nicotine) and NMDA receptor antagonists (MK-801) and larger than that observed with dopamine receptor agonists (pergolide), nNOS inhibitors (7-NI) (O'Neill et al., unpublished results). In contrast, we failed to see any protection with AMPA antagonists (LY293558) and immunophilins (FK-506) under similar conditions (O'Neill et al., unpublished results). In rats bearing a partial lesion of the nigrostriatal tract induced by intrastriatal 6-OHDA infusion, LY379268 provided significant protection in the striatum against the decline in TH immunoreactivity, although no significant protection of the dopaminergic neurones in the SNc was apparent.

Our laboratory has previously reported that Group II mGlu receptor activation by LY379268 provided protection against glutamate-mediated neurotoxicity in cortical neurones in vitro (Kingston et al., 1999) and against global ischaemia (Bond et al., 2000). It is clear that the protection in these situations could be mediated by either presynaptic

activation of mGluR2 to reduce glutamate release or by activation of mGluR3 on glia. It has been reported that activation of mGluR3 releases a heat sensitive neuroprotective factor (Bruno et al., 1997), which was sensitive to protein synthesis inhibitors and later identified as TGFβ1 (Bruno et al., 1998). More recently, it has be reported that activation of Group II mGluRs can protect against MPP^+ neurotoxicity and probably via the increases in BDNF observed since the effect was inhibited by the protein synthesis inhibitor cycloheximide (Matarredona et al., 2001). These neurotrophic factor studies were carried out using DCG-IV, which is not selective for Group II mGlu receptors. However, it is well established that growth factors such as BDNF and GDNF can protect (Beck et al., 1995; Tomac et al., 1995) and enhance recovery of the nigrostriatal dopaminergic system in MPTP-lesioned animals (Gash et al., 1996). Therefore, it is possible that the limited neuroprotection seen in the present studies may be due to restricted increases in neurotrophic factor production, but further studies are required to investigate this. In addition, it would be useful to evaluate selective mGluR2 and mGluR3 agonists in these models, but to date these are not available.

5. Conclusions

In conclusion, these data demonstrate that Group II mGlu receptors play some role in alleviating akinesia in the rat. However, their role is complex and systemic administration appears to alter several neuronal circuits or neurotransmitter systems that negate any beneficial effect. The data also demonstrate some neuroprotective actions of Group II mGlu receptor activation. These results are encouraging and, although further testing with more selective molecules and in other models is required, these results suggest that mGluRs may offer a nondopaminergic approach to the treatment of PD.

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