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Research Paper

Exendin-4 treatment enhances L-DOPA evoked release of striatal dopamine and decreases dyskinetic movements in the 6-hydoxydopamine lesioned rat

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Keywords
6-hydroxydopamine; dopamine release; exendin-4; L-DOPA induced dyskinesias; Parkinson's disease

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Abstract

Objectives To determine whether the glucagon-like 1 peptide analogue exendin-4 (EX-4) augments the neurochemical effects of a single L-DOPA treatment and whether EX-4 can decrease L-DOPA induced dyskinesias (LIDS).

Methods Rats were lesioned with 6-hydroxydopamine (6-OHDA) and 7 days later given EX-4 for 7 days. The following day, rats were given L-DOPA and extracellular dopamine was measured. The animals were then killed to determine tissue dopamine. To study LIDS, EX-4 and/or L-DOPA were co-administered daily, 7 days after 6-OHDA. LIDS were determined on Days 2, 4, 8, 12 and 16 prior to neurochemical assessment.

Key findings EX-4 reduced 6-OHDA induced damage. Acute effects of L-DOPA were potentiated by EX-4 in lesioned rats. Treatments with EX-4 caused a progressive reduction in LIDS.

Conclusions EX-4 treatment potentiates the effects of a single dose of L-DOPA. This augmentation indicates that lower L-DOPA doses might be used to the same effect in patients. The reduction in LIDS suggests that co-treatment with EX-4 could allow the use of L-DOPA with fewer side-effects and possibly therefore allow earlier introduction of L-DOPA in the clinic.

Introduction

Treatment with the dopamine (DA) precursor, L-dihydroxyphenylalanine (L-DOPA), is currently the mainstay therapy used in Parkinson's disease (PD).[1] When first introduced into clinical practice, L-DOPA was regarded as a breakthrough therapy, and its continuing use is a testimony to its utility in PD. However, this continuing reliance on L-DOPA is indicative of the failure to deliver a better alternative in over 40 years. L-DOPA treatment is associated with a number of side-effects such as dyskinesias and sometimes psychiatric phenomena,[2–4] while the efficacy of L-DOPA treatment declines with time.[1] Nevertheless, the majority of PD patients are ultimately treated with L-DOPA,[5] although this treatment is often delayed due to concerns about its side-effects.[5] Thus, there is a strong case for therapeutic strategies wherein L-DOPA doses may be reduced or their side-effects ameliorated without loss of therapeutic efficacy over motor control. Ideally, these should be based upon approaches aimed to arrest further development of the lesion or even reverse it.

We have recently reported that the glucagon-like peptide 1 (GLP-1) receptor agonist exendin-4 (exenatide; EX-4) is highly effective in reversing key deficits in preclinical rodent models of PD,[6] an effect also observed by Bertilsson *et al.*[7] and confirmed more recently by Li *et al.*[8] using knockout mice for the GLP-1 receptor (GLP-1R). In rats with moderate/severe[6] or severe[7] degeneration of the nigrostriatal dopaminergic system EX-4 appears to, at the very least, arrest the damage following neurotoxin treatment such as apomorphine induced turning, striatal and nigral DA depletion, reduction in nigral tyrosine hydroxylase staining and loss of vesicular monoamine transporter 2 positive cells.[6,7] Importantly, these effects can be seen when EX-4 is given once the toxin induced lesion is established.[6,7] This is significant since diagnosis of PD is currently only made once very considerable damage to the nigrostriatal pathway has occurred and therefore any advance in current therapy needs to account for this. Ideally, any therapy should be able to

arrest or better still reverse the degeneration which is on-going in PD, actions that appear possible for EX-4.

We studied the effects of acute EX-4 on L-DOPA or apomorphine induced rotations, tissue and extracellular DA in the 6-hydroxydopamine (6-OHDA) lesioned rat. We have also examined the effects of chronic EX-4 given concomitantly with L-DOPA on L-DOPA induced dyskinesias (LIDS) in lesioned rats. A significant novel beneficial effect of EX-4 on LIDS would be of great interest and potential benefit as currently there are no effective means of ameliorating these debilitating side-effects.

Methods and Materials

Materials

L-DOPA, desmethylimipramine, EX-4, pargyline, benserazide, 6-OHDA and apomorphine hydrochloride were all obtained from Sigma, UK. Apomorphine, L-DOPA and 6-OHDA were dissolved in 1.0% w/v ascorbic acid. All drugs, apart from 6-OHDA were injected in a volume of 0.1 ml/100 g bodyweight. All other reagents were of Analar or high-performance liquid chromatography (HPLC) grade.

Animals and surgical procedures

Experiments were carried out in accordance with the Animals (Scientific Procedures) Act UK (1986) and under the governance of the School of Pharmacy Animal Ethics Committee. Male Wistar rats (210–240 g) were housed in groups and had access to food and water *ad libitum*. Care was taken to minimize animal usage and tissue was typically used in several different assay paradigms. Before surgery, animals to be lesioned with 6-OHDA received pargyline (50 mg/kg, i.p.) and desmethylimipramine (25 mg/kg, i.p.) in order to maximize the selectivity of the toxin for dopaminergic neurons. Animals were anaesthetized with isofluorane (4% for induction, 1.5% for maintenance), secured in a stereotaxic frame (David Kopf, USA) and given injections of 6-OHDA (Sigma, Poole, UK) (8 μg/4 μl of saline with 1% ascorbic acid) injected into the right medial forebrain bundle (from bregma in mm; A −4.3, L 1.4 and V 8.2). Seven days later, rats were administered EX-4 (0.1 or 0.5 μg/kg i.p, twice daily) dissolved in 0.9% saline for a period of 7 days, then given a single injection of L-DOPA. This time point was chosen since at the point of EX-4 intervention the lesion was still progressing,[5] as is the case in PD patients. For chronic studies, rats were treated as above except that 7 days after 6-OHDA animals were administered EX-4 + L-DOPA (10 mg/kg, once daily) or saline for a period of 16 days, during which they were tested for LIDS on Days 0, 2, 4, 8, 12 and 16 as described by Lane and Dunnett,[9] after which they were processed as described below. In all experiments, both acute and chronic benserazide (12.5 mg/kg) was given prior to L-DOPA. Benserazide alone was not found to alter any of the parameters studied in this investigation and these data are therefore not presented.

Assessment of nigrostriatal lesion severity following apomorphine/L-DOPA challenge

All animals received an apomorphine challenge (0.05 mg/kg s.c.) in order to determine the presence of a lesion, with a minimum of 15 rotations in a 120-s set as acceptable.[6,10] Animals were placed in a circular test arena and following a short period of acclimatization, injected with the dopaminergic agonist. Contralateral turns were noted 20 min after injection and recorded over a 120-s observation period. Only complete, 'tight' turns were recorded. We have tested the value of observing rats for longer periods of time (up to 60 min) and recording rotations. Although, the absolute number of rotations was obviously considerably higher using this protocol, the relative difference between groups was virtually identical to sampling for the much shorter time period and the overall methodology was far more time efficient. To study the effects of L-DOPA, rats were administered benzserazide (12.5 mg/kg i.p.) in order to reduce metabolism of L-DOPA in the periphery and increase its bioavailability to the central nervous system (CNS), and 30 min later L-DOPA (10 or 30 mg/kg i.p.) was injected. Rats were then treated exactly as for apomorphine-injected groups and the contralateral rotations counted.

In-vivo microdialysis

Following apomorphine testing, rats were implanted with concentric dialysis probes of a construction previously described with an active membrane length of 4 mm.[11] Probes were bilaterally implanted into each striatum (from bregma in mm; A +0.2, L 3.0, and V 8.2; co-ordinates from Paxinos and Watson[12] and the following day perfused with an artificial cerebrospinal fluid solution (composition in mM: 2.5 KCl; 125 NaCl; 1.18 $MgCl_2$; 1.26 $CaCl_2$) as previously used,[11] but without addition of the 5-HT reuptake inhibitor citalopram. Following a 1-h equilibration period, four 30-min 'basal' samples were collected before injection of L-DOPA or vehicle. Microdialysis was not performed on rats chronically treated with L-DOPA as the resulting LIDS led to repeated problems with the dialysis leads.

DA assay

Animals received pargyline (50 mg/kg) 30 min before being killed. Brains were removed and the striata were dissected and homogenized in ice-cold phosphate buffer (pH 7.4). All homogenates were treated with 0.2 M perchloric acid (1 : 10, w/v) containing ascorbic acid (0.2 μM) and EDTA (0.2 mM), to precipitate cell debris. These were then centrifuged at 9000 *g* for 15 min at 4°C, supernatants passed through a

syringe filter (10 μm pore size) and whole-tissue dopamine levels estimated using HPLC with electrochemical detection.[11] In the case of dialysis samples, dialysates were analysed using HPLC with electrochemical detection[11] as above except that since these samples were already free of proteins or particulate matter, they were analysed directly after collection with no further processing.

Statistical analysis

Data obtained from apomorphine challenge and whole-tissue DA assay studies were expressed as mean values ± SEM. Data were subjected to one-way or two-way analysis of variance to identify overall trends, with a post-hoc Bonferroni's multiple comparison test used to establish significant differences between the groups. Statistical analysis was performed using a proprietary software package (GraphPad Prism, http://www.graphpad.com/prism/prism.htm). The numbers of animals used in the experiments are detailed in the figure legends. In all cases, comparisons were made with respect to toxin/vehicle values. Statistical significance was set at $P < 0.05$.

Results

Apomorphine induced circling is regarded as an indication of nigrostriatal lesion severity and thus an attenuation is predictive of potential antiparkinsonian activity. Our findings are consistent with previous observations[6] revealing that contralateral circling was clearly evident in 6-OHDA lesioned rats, being dose-dependently attenuated by EX-4 (Figure 1), with lesions already evident 7 days after injection when EX-4 treatment commenced.[10] Administration of L-DOPA led to intense contralateral circling activity, which was also attenuated by EX-4 treatment (Figure 1).

Measurement of striatal tissue DA revealed a dramatic loss of this transmitter in 6-OHDA treated rats (Figure 2). Administration of L-DOPA elevated striatal tissue DA content and this was dose-dependently augmented by prior treatment with EX-4, and was significantly greater than control values at the higher EX-4 dose following chronic L-DOPA treatment (Figure 2).

In order to evaluate the effect of EX-4 on extracellular DA in the striatum, we used microdialysis in freely moving rats. We have assumed that extracellular DA represents a functional index of DA release and this is supported by our observation that 1 μM tetrodotoxin infusion decreased dialysate DA by over 90% in sham-treated rats (data not shown). Although rats were bilaterally implanted with dialysis probes, we have only presented DA values from the lesioned side since values from the contralateral side were virtually identical to sham-treated rats for any given procedure, apart from 6-OHDA treatment itself. 6-OHDA greatly reduced

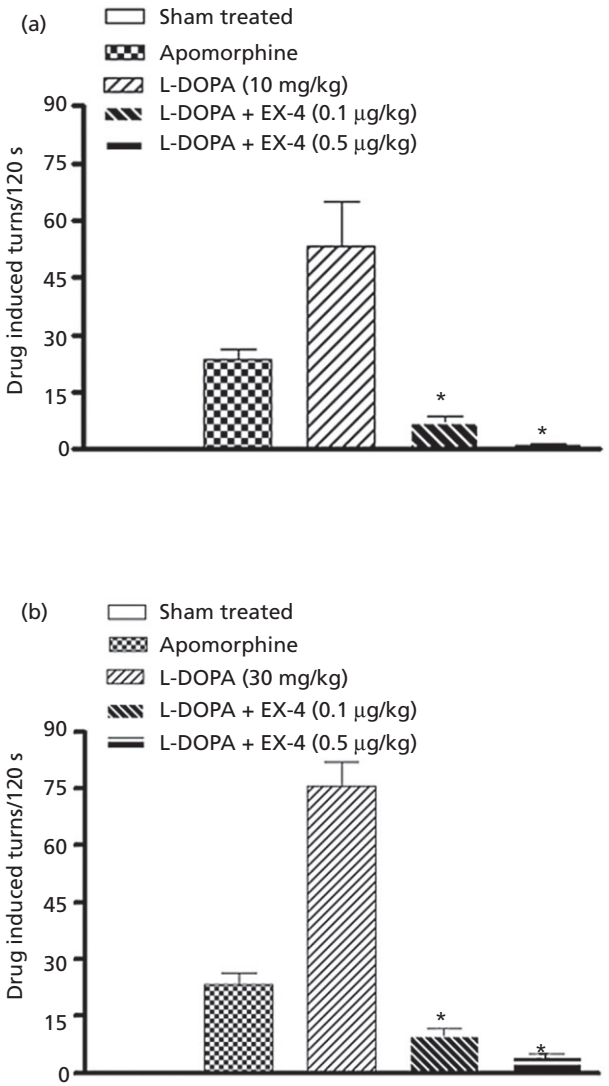

Figure 1 Effect of exendin-4 (EX-4) (0.1 and 0.5 μg/kg) on apomorphine or L-DOPA induced rotational behaviour in 6-hydroxydopamine lesioned rats. Rats were given benserazide (12.5 mg/kg) and 30 min later L-DOPA was given at 10 mg/kg (a) or 30 mg/kg (b). At 7 days after toxin injection, EX-4 was administered twice daily for 7 days. Circling was measured for 120 s at 15 min after apomorphine or L-DOPA injection. One-way analysis of variance values were 15.69, $P < 0.001$ (a) and 18.54, $P < 0.001$ (b). *$P < 0.01$, significant differences compared with apomorphine or L-DOPA treated groups using Bonferroni's multiple comparison test ($n = 6$ per group).

extracellular DA (Figure 3). L-DOPA evoked a dose-dependent increase in extracellular DA that was itself dose-dependently augmented when rats were treated with EX-4 (Figure 3). This restoration of basal and L-DOPA stimulated DA release by EX-4 suggests a functional basis upon which motor movement (circling behaviour) is normalized in apomorphine or L-DOPA treated rats (Figures 1 and 3). In order to establish that the effects of EX-4 were the result of repeated

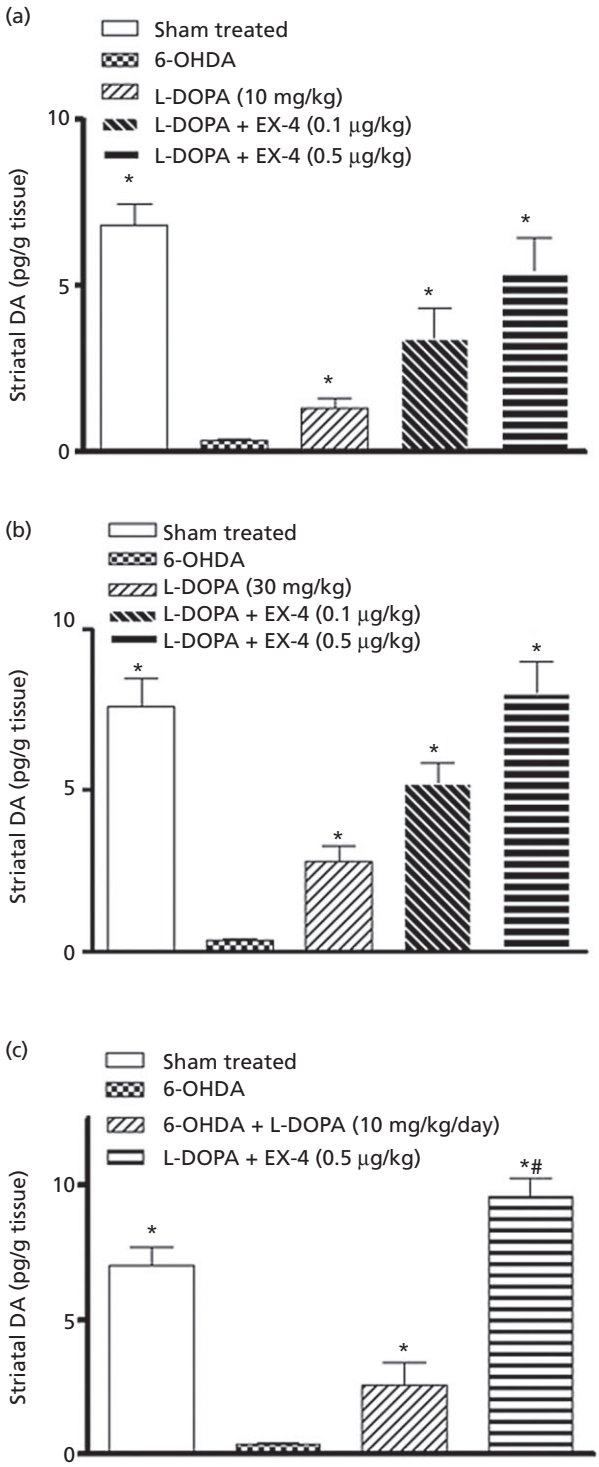

Figure 2 Effect of exendin-4 (EX-4) on striatal tissue dopamine (DA) content in 6-hydroxydopamine (6-OHDA) lesioned rats given acute L-DOPA at 10 mg/kg (a) and 30 mg/kg (b) or chronic L-DOPA at 10 mg/kg per day for 16 days (c). L-DOPA injection was always preceded by 12.5 mg/kg benserazide. At 7 days after toxin injection, EX-4 was administered twice daily for 7 days. One-way analysis of variance values were 14.98, $P < 0.001$ (a), 16.67, $P < 0.001$ (b) and 29.42, $P < 0.001$ (c). $*P < 0.01$, significant differences compared with 6-OHDA only injections and $\#P < 0.01$ compared with L-DOPA + 6-OHDA injection using Bonferroni's multiple comparison test ($n = 6$ per group).

rather than an acute effect of the peptide, dialysis was performed in rats after a single EX-4 injection. Under these conditions the effect of L-DOPA on extracellular DA was not different to that seen in rats treated with 6-OHDA alone, indicating that an extended period of EX-4 treatment is required (Figure 3).

Chronic (16 day) treatment with L-DOPA led to the appearance and the progressive increase in LIDS in 6-OHDA treated rats (Figure 4). LIDS developed rapidly, appearing by Day 2 and peaking by Day 8 of L-DOPA treatment, and remained at a similar level of intensity thereafter (Figure 4), which is consistent with previous observations by others.[9] LIDS was not observed in unlesioned rats. Rats co-treated with L-DOPA and EX-4 displayed an initial rise in LIDS similar to that seen after L-DOPA treatment alone (Figure 4), but after Day 4 the group co-treated with EX-4 showed a progressive reduction in the intensity of LIDS (Figure 4).

Discussion

Since the introduction around 50 years ago of L-DOPA for the symptomatic treatment of PD, the successful development of treatments from theory to clinical success has been extremely sparse. L-DOPA combined with the inhibition of peripheral DOPA-decarboxylase produces a marked improvement in motor symptoms in most patients with PD. However, over time, patients treated with L-DOPA develop a number of side-effects, such as dyskinesias and substantial fluctuations in motor function.[1,13,14] The mechanisms leading to these dyskinesias are not fully known but appear to be linked to the severity of the disease and the duration and dose of L-DOPA used.[2] Treatments have been studied that may reduce nigrostriatal degeneration or restore function such as glial cell derived neurotrophic factor (GDNF)[15,16] or direct stimulation of DA receptors.[17] However, the progressive nature of PD constitutes a clear barrier to the ultimate success of any symptomatic treatment strategy as these are likely to require periodic increases in drug dose, although ameliorating significant side-effects provides a means to prolong the useful lifespan of a drug. A number of non-pharmacological interventions have been studied including deep brain stimulation, which has been shown to produce impressive results in some patients,[18,19] but the numbers for whom this treatment is potentially available are always likely to be severely limited by the fact that it is a neurosurgical procedure. This limitation would also appear to hold true for gene therapy, stem cell or tissue transplant introduction into the CNS, at least for the foreseeable future.[16,20,21] This is also the case for direct delivery of factors unable to cross the blood

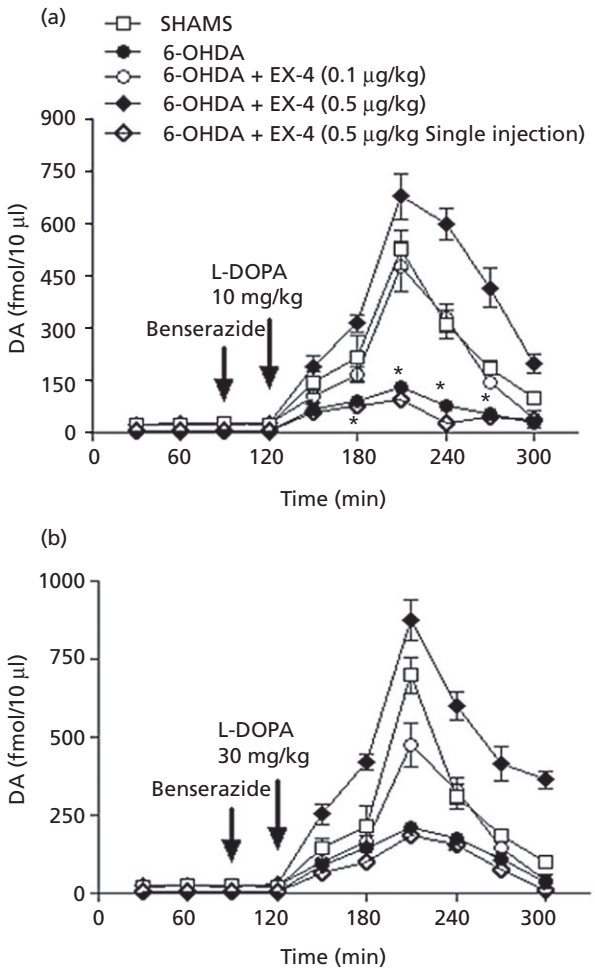

Figure 3 Effect of exendin-4 (EX-4) (0.1 and 0.5 μg/kg) on striatal extracellular dopamine (DA) content in 6-hydroxydopamine (6-OHDA) lesioned rats given L-DOPA at 10 mg/kg (a) and 30 mg/kg (b). At 7 days after toxin injection, EX-4 was administered twice daily for 7 days after toxin injection. Injection of benserazide is indicated by an arrow. For single injections of EX-4, the drug was administered immediately after benserazide on the day of microdialysis. Two-way analysis of variance values were 44.94, $P < 0.001$ between treatments and 147.3, $P < 0.001$ over time (a) and 109.0, $P < 0.001$ between treatments and 153.4, $P < 0.001$ over time (b). $P < 0.01$, significant differences compared with groups not given 6-OHDA only, while $P < 0.01$, denotes significance compared with groups not receiving EX-4. Post-hoc analysis using Bonferroni's multiple comparison test ($P < 0.01$, $n = 6$ per group).

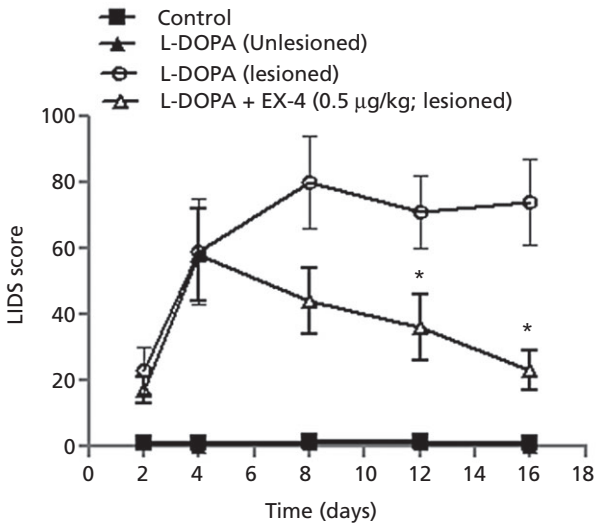

Figure 4 Effect of exendin-4 (EX-4) on L-DOPA induced dyskinesias (LIDS) following repeated administration of L-DOPA. At 1 week after 6-hydroxydopamine (6-OHDA) treatment, rats were administered EX-4 (0.5 μg/kg, twice daily), L-DOPA (10 mg/kg per day) or vehicle. Benserazide (12.5 mg/kg) was administered 30 min before L-DOPA injection. Immediately after L-DOPA treatment, rats were scored for LIDS at different time points up to 16 days. Two-way analysis of variance values were 62.48, $P < 0.001$ between treatments and 5.43, $P < 0.001$ over time. Note that with no incidence of LIDS, sham-treated and 6-OHDA only lines are superimposed. Post-hoc analysis using Bonferroni's multiple comparison test ($P < 0.01$, $n = 6$ per group).

brain barrier such as GDNF.[22] This appears to leave small molecule pharmacology as one of the more realistic avenues of success, although this approach has been traditionally symptomatic rather than restorative. EX-4 is a comparatively large molecule but it readily enters the CNS,[23] and is highly effective in reversing cardinal deficits seen in preclinical models of PD. Since diagnosed PD patients are highly likely to be on L-DOPA medication, the interaction between these drugs is likely to be a significant consideration.

Extracellular levels of DA are drastically reduced in 6-OHDA lesioned rats.[6,24,25] However, there are disparities between reports on the effects of L-DOPA on extracellular DA levels in the striatum of lesioned or non-lesioned rats. Some authors have reported a substantially greater L-DOPA-evoked DA release in lesioned rats, presumably due to an upregulation of DA synthetic capacity in the remaining dopaminergic nerve terminals. Other studies, including the present investigation, have found DA levels to be more substantially increased in intact rather than lesioned rats.[24,26] This may be due to the extent of the lesion, leading to a loss of DA terminals that may be too substantial to overcome the compensatory preysnaptic strategies within the striatum, such as enzyme upregulation. Perhaps the key acute observation here, however, is that EX-4 dose-dependently increased the amount of extracellular DA dopamine in response to a given L-DOPA dose. We have previously found EX-4 to cause a restoration of, or to arrest further loss of tyrosine hydroxylase positive cells in the substantia nigra of lesioned rats[6] and presume that this provides a means for a more substantial conversion of L-DOPA to DA in the nigrostriatal cells. This normalisation of dopaminergic function presumably underlies the reduced circling behaviour following either

apomorphine or L-DOPA challenge in lesioned rats. The progressive reduction in LIDS seen in the present study may therefore reflect a restoration of functional dopaminergic transmission within the nigrostriatal system, which presumably leads to a more normal release of DA. It is also possible that systems other than the DA system within the striatum are modulated by EX-4 treatment. Interestingly, it has recently been reported that EX-4 treatment leads to neuroprotection in an experimental model of ischaemia in gerbils,[27] while at the same time evoking progressive increases in GLP-1R expression in hippocampal astrocytes and GABAergic interneurons.[27] The striatum is predominately composed of GABAergic neurons and it is possible that the effects seen following EX-4 may be secondary to altered transmission of other neurotransmitter systems than GLP-1 alone. Indeed, a progressive neurochemical change following EX-4, such as altered GLP-1R expression, could account for the failure of EX-4 to immediately inhibit LIDS (Figure 4).

Should EX-4 meet with clinical success in PD patients, those currently on L-DOPA may be able to lower the dose of this drug to achieve a given motor response. This could be accompanied by, at the least, a decrease in L-DOPA induced side-effects. In addition, however, it may be that even at the same L-DOPA dose dyskinesias may be decreased by EX-4, which would represent a great improvement in patient quality of life. Moreover, a diminution in L-DOPA induced side-effects could allow the introduction of this drug at an earlier time after diagnosis of PD since side-effects are a significant factor in the delay in treating with L-DOPA.

Conclusions

We have observed that EX-4 treatment potentiates the effects of an acute dose of L-DOPA. This augmentation indicates that lower L-DOPA doses might be used to the same effect in patients. In addition, the reduction in LIDS suggests that co-treatment with EX-4 could allow the use of L-DOPA with fewer side-effects. This would achieve clear benefits for individual patients and could possibly allow earlier introduction of L-DOPA in the clinic since LIDS are a limiting factor in the decision to treat with L-DOPA. Since EX-4 is in current use, has a moderate side-effect profile and does not induce hypoglycaemia or significant weight loss in non-diabetic individuals,[28] it would seem appropriate to test its utility in Parkinsonian patients without delay.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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References

1. Marsden CD, Parkes JD. Success and problems of long-term levodopa therapy in Parkinson's disease. *Lancet Neurol* 1977; 1: 345–349.
2. Nutt J. Levodopa-induced dyskinesia: review, observations, and speculations. *Neurology* 1990; 37: 340–345.
3. Calabresi P *et al.* Levodopa-induced dyskinesia: a pathological form of striatal synaptic plasticity? *Ann Neurol* 2000; 47(Suppl. 1): S60–S69.
4. Olanow CW, Obeso JA. Pulsatile stimulation of dopamine receptors and levodopa-induced motor complications in Parkinson's disease: implications for the early use of COMT inhibitors. *Neurology* 2000; 55(Suppl. 1): 56–63.
5. Hauser RA. Early pharmacologic treatment in Parkinson's disease. *Am J Manag Care* 2010; 16: 100–107.
6. Harkavyi A *et al.* Glucagon-like peptide 1 receptor stimulation reverses key deficits in distinct rodent models of Parkinson's disease. *J Neuroinflammation* 2008; 5: 19.
7. Bertilsson G *et al.* Peptide hormone exendin-4 stimulates subventricular zone neurogenesis in the adult rodent brain and induces recovery in an animal model of Parkinson's disease. *J Neurosci Res* 2008; 86: 326–338.
8. Li Y *et al.* GLP-1 receptor stimulation preserves primary cortical and dopaminergic neurons in cellular and rodent models of stroke and Parkinsonism. *Proc Natl Acad Sci USA* 2009; 106: 1285–1290.
9. Lane EL, Dunnett SB. Pre-treatment with dopamine agonists influence l-dopa mediated rotations without affecting abnormal involuntary movements in the 6-OHDA lesioned rat. *Behav Brain Res* 2010; 213: 66–72.
10. Abuirmeileh A *et al.* The corticotrophin-releasing factor-like peptide urocortin reverses key deficits in two rodent models of Parkinson's disease. *Eur J Neurosci* 2007; 26: 417–423.
11. Biggs CS *et al.* Regional effects of sodium valproate on extracellular concentrations of 5-hydroxytryptamine, dopamine and their metabolites in the rat brain: an in vivo microdialysis study. *J Neurochem* 1992; 59: 1702–1708.
12. Paxinos G, Watson C. *The Rat Brain in Stereotaxic Coordinates*, 4th edn. New York: Academic Press, 1998.
13. Rascol O *et al.* Idazoxan, an alpha-2 antagonist, and L-DOPA-induced dyskinesias in patients with Parkinson's disease. *Mov Disord* 2001; 16: 708–713.
14. Rajput AH *et al.* Clinical-pathological study of levodopa complications. *Mov Disord* 2002; 17: 289–296.

15. Choi-Lundberg DL *et al*. Dopaminergic neurons protected from degeneration by GDNF gene therapy. *Science* 1997; 275: 838–841.
16. Akerud P *et al*. Neuroprotection through delivery of glial cell line-derived neurotrophic factor by neural stem cells in a mouse model of Parkinson's disease. *J Neurosci* 2001; 21: 8108–8118.
17. Olanow CW *et al*. Continuous dopamine-receptor treatment of Parkinson's disease: scientific rationale and clinical implications. *Lancet Neurol* 2006; 5: 677–687.
18. Vaillancourt DE *et al*. Effects of deep brain stimulation and medication on bradykinesia and muscle activation in Parkinson's disease. *Brain* 2004; 127: 491–504.
19. Vaillancourt DE *et al*. Effects of deep brain stimulation and medication on strength, bradykinesia, and electromyographic patterns of the ankle joint in Parkinson's disease. *Mov Disord* 2006; 21: 50–58.
20. Bensadoun JC *et al*. Lentiviral vectors as a gene delivery system in the mouse midbrain: cellular and behavioral improvements in a 6-OHDA model of Parkinson's disease using GDNF. *Exp Neurol* 2000; 164: 15–24.
21. Barker RA. Repairing the brain in Parkinson's disease: where next? *Mov Disord* 2002; 17: 233–241.
22. Gill SS *et al*. Direct brain infusion of glial cell line-derived neurotrophic factor in Parkinson disease. *Nat Med* 2003; 9: 589–595.
23. Kastin AJ, Akerstrom V. Entry of exendin-4 into brain is rapid but may be limited at high doses. *Int J Obes* 2003; 27: 313–318.
24. Dethy S *et al*. Pergolide potentiates L-DOPA-induced dopamine release in rat striatum after lesioning with 6-hydroxydopamine. *J Neural Transm* 1999; 106: 145–158.
25. Kannari K *et al*. Reserpine pretreatment prevents increases in extracellular striatal dopamine following L-DOPA administration in rats with nigrostriatal denervation. *J Neurochem* 2000; 74: 263–269.
26. Jonkers N *et al*. MK801 influences L-DOPA-induced dopamine release in intact and hemi-parkinson rats. *Eur J Pharmacol* 2000; 407: 281–291.
27. Lee CH *et al*. Ischaemia-induced changes in glucagon-like peptide-1 and neuroprotective effect of its agonist, exendin-4, in experimental transient cerebral ischaemia. *J Neurosci Res* 2011; 89: 1103–1113.
28. Byetta Prescribing Information. Indianapolis N: Eli Lilly and Company. 2010. Available at http://pi.lilly.com/us/byetta-pi.pdf.