Nicotinic Receptor Agonists Reduce L-DOPA–Induced Dyskinesias in a Monkey Model of Parkinson's Disease

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ABSTRACT

Abnormal involuntary movements or dyskinesias are a serious complication of long-term L-DOPA treatment of Parkinson's disease, for which there are few treatment options. Accumulating preclinical data show that nicotine decreases L-DOPA–induced dyskinesias (LIDs), suggesting that it may be a useful antidyskinetic therapy for Parkinson's disease. Here, we investigated whether nicotinic acetylcholine receptor (nAChR) agonists reduced LIDs in nonhuman primates. We first tested the nonselective nAChR agonist 1, 6,7,8,9-tetrahydro-6,10-methano-6H-pyrazino[2,3-h][3]benzazepine (varenicline), which offers the advantage that it is approved by the U.S. Food and Drug Administration for use in humans. 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)–lesioned monkeys ($n = 23$) were first administered L-DOPA/carbidopa (10/2.5 mg/kg) twice daily 5 days/week until stably dyskinetic. Oral varenicline (0.03–0.10 mg/kg) decreased LIDs ~50% compared with

vehicle-treated monkeys, whereas nicotine treatment (300 μ g/ml in drinking water) reduced LIDs by 70% in a parallel group of animals. We next tested the selective $\alpha 4\beta 2^*/\alpha 6\beta 2^*$ nAChR agonist TC-8831 [3-cyclopropylcarbonyl-3,6-diazabicyclo[3.1.1]heptane] on LIDs in the same set of monkeys after a 10-week washout. We also tested TC-8831 in another set of MPTP-lesioned monkeys ($n = 16$) that were nAChR drug-naïve. Oral TC-8831 (0.03–0.3 mg/kg) reduced LIDs in both sets by 30–50%. After a washout period, repeat TC-8831 dosing led to a greater decline in LIDs (60%) in both sets of monkeys that was similar to the effect of nicotine. Tolerance to any nAChR drug did not develop over the course of the study (3–4 months). NACHR drug treatment did not worsen parkinsonism or cognitive ability. These data suggest that nAChR agonists may be useful for the management of dyskinesias in L-DOPA–treated Parkinson's disease patients.

Introduction

Although L-DOPA is one of the most effective therapies for Parkinson's disease, its continued use leads to abnormal involuntary movements or dyskinesias that may severely affect the quality of life of Parkinson's disease patients (Cenci and Konradi, 2010; Brochie and Jenner, 2011; Carta and Bezard, 2011; Fisone and Bezard, 2011; Iravani and Jenner, 2011; Prashanth et al., 2011; Rascol et al., 2011; Poewe et al., 2012; Huot et al., 2013). As there are few treatment options to manage L-DOPA–induced dyskinesias (LIDs), novel therapeutic approaches are critical. The observation that the nicotinic cholinergic system shares a close anatomical relationship with the striatal dopaminergic system and that nicotinic acetylcholine receptor (nAChR) activation modulates dopaminergic function prompted work to investigate the effect of nicotine on LIDs.

Nicotine treatment decreased LIDs in parkinsonian monkey, rat, and mouse models, suggesting that it may represent a useful treatment approach (Quik et al., 2007; Bordia et al., 2008, 2010; Huang et al., 2011a). Moreover, various modes of nicotine administration successfully attenuated LIDs, including injection, oral treatment, and minipump infusion, some of which could readily be used in the clinic. Tolerance to the effect of nicotine did not develop in any species with study periods up to 6 months (Quik et al., 2007; Bordia et al., 2008, 2010; Huang et al., 2011a).

The primary nAChRs in the basal ganglia are the $\alpha 6\beta 2^*$ and $\alpha 4\beta 2^*$ subtypes, with a smaller subset of $\alpha 7$ nAChRs (Millar and Gotti, 2009; Gotti et al., 2010; Quik and Wonnacott, 2011). Studies in parkinsonian null mutant mice suggest that nicotine may reduce LIDs by acting at one or several of these nAChR populations. The use of $\beta 2$ nAChR subunit knockout mice, which lack both the $\alpha 4\beta 2^*$ and $\alpha 6\beta 2^*$ nAChR subtypes, showed that LIDs develop to a lesser extent in such mice, and that nicotine no longer had an antidyskinetic effect (Huang et al., 2011a). Similar results were obtained with $\alpha 6$ nAChR subunit knockout mice (Quik et al., 2012), as well as $\alpha 4$ nAChR

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ABBREVIATIONS: A85380, 3-[(2S)-2-azetidinylmethoxy-5-iodo-pyridine dihydrochloride; ANOVA, analysis of variance; CNS, central nervous system; LIDs, L-DOPA–induced dyskinesias; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; nAChR, nicotinic acetylcholine receptor; OHDA, 6-hydroxydopamine; TC-8831, 3-cyclopropylcarbonyl-3,6-diazabicyclo[3.1.1]heptane; *, the possible presence of other nicotinic subunits in the receptor complex.

subunit knockout mice (Quik et al., 2013b). These combined data indicate that the $\alpha 4$, $\alpha 6$, and $\beta 2$ nAChR subunits (that is, $\alpha 4\beta 2^*$ and $\alpha 6\beta 2^*$ nAChR populations) play a role in LIDs. The use of $\alpha 7$ nAChR knockout mice showed that this subtype also modulated the development of LIDs, although in a manner distinct from that mediated by $\alpha 4\beta 2^*$ and $\alpha 6\beta 2^*$ nAChRs (Quik et al., 2013b). $\alpha 7$ nAChR subunit deletion increased baseline LIDs, suggesting that this subtype exerts an inhibitory effect. The development of LIDs may thus involve a concerted action of acetylcholine at $\alpha 4\beta 2^*$, $\alpha 6\beta 2^*$, and $\alpha 7$ nAChRs.

Pharmacological studies with nAChR agonists and antagonists also point to an involvement of $\alpha 4\beta 2^*$ and $\alpha 6\beta 2^*$ nAChRs in LIDs. nAChR agonists, including 1, 6,7,8,9-tetrahydro-6,10-methano-6H-pyrazino[2,3-h][3]benzazepine (varenicline), A85380, and compounds, such as TC-8831 (Targacept, Inc., Winston-Salem, NC), that act at both $\alpha 4\beta 2^*$ and $\alpha 6\beta 2^*$ subtypes decreased the occurrence of LIDs in 6-OHDA-lesioned rats (Huang et al., 2011b; Quik et al., 2013a). In addition, a recent study showed that TC-8831 reduced LIDs in parkinsonian monkeys (Johnston et al., 2013).

The objective of the current study was to determine whether the general nAChR agonist varenicline, which is approved by the U.S. Food and Drug Administration for use in humans for smoking cessation, reduces LIDs in a nonhuman primate model of Parkinson's disease. This drug offers the advantage that it could readily be investigated in the clinic for the treatment of LIDs (Rollema et al., 2007; Cahill et al., 2011). The second objective was to extend studies with the selective central nervous system (CNS) nAChR agonist TC-8831, as previous work demonstrated a small decline in LIDs which did not persist with continued dosing (Johnston et al., 2013). TC-8831 offers the advantage that there is little interaction at peripheral nervous system receptors—that is, the cardiovascular, gastrointestinal, and other autonomic systems. We also tested the effect of the agonists on a memory task, as nAChR agonists have been shown to enhance various aspects of memory and/or attention in clinical trials (Rhodes et al., 2012; Shim et al., 2012). Results show that both varenicline and TC-8831 reduced LIDs by 30–70% with no development of tolerance over several months. Furthermore, TC-8831 reduced LIDs as effectively as nicotine with repeated use. These data suggest that nAChR agonists such as TC-8831 may be useful in the treatment of LIDs that arise in Parkinson's disease.

Materials and Methods

Animals. Squirrel monkeys (*Saimiri sciureus*), purchased from Worldwide Primates (Miami, FL), were quarantined after arrival for a 1-month period, as required by California state regulations. The monkeys were of both sexes and weighed between 0.6 and 1.2 kg. The animals were housed under a 12-hour light/dark cycle, in a temperature- and humidity-controlled room. They were provided with water ad libitum and fed monkey chow, fruits, and vegetables. All studies were performed according to the U.S. National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee.

MPTP Administration and Parkinsonian Ratings. After quarantine, the monkeys were individually housed and trained in a variety of motor tasks to allow for future assessment of parkinsonism (Quik et al., 2007). Parkinsonism was scored on a scale from 0 (normal) to 4 (severe) for each category, and included spatial

hypokinesia (use of available cage space), body bradykinesia (slowness in body movement), balance (ability to maintain posture), freezing, tremor, and left and right manual dexterity. After training, 2.0 mg/kg 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP; Sigma-Aldrich, St. Louis, MO) dissolved in saline was injected subcutaneously (Quik et al., 2007). MPTP injection was repeated once (1.9 mg/kg s.c.) 3–4 weeks later, and parkinsonism was rated once weekly (Monday) before and 1–2 hours after L-DOPA administration throughout the course of the study.

L-DOPA Treatment and Dyskinesia Ratings. All monkeys were gavaged two times per day at 3.5-hour intervals with L-DOPA (10 mg/kg) and carbidopa (2.5 mg/kg) for 5 days per week for at least 4 weeks, such that they were stably dyskinetic. During the course of L-DOPA treatment, monkeys were given only fruits and vegetables in the morning to allow optimal absorption of L-DOPA. Monkey chow, fruits, and vegetables were given in the afternoon 4 hours after the second L-DOPA dose. The monkeys were video recorded for the entire day (8:00 AM to 4:00 PM), with baseline recording performed Tuesday, Wednesday, and Thursday from 8:00 to 8:30 AM before the first dose of L-DOPA. L-DOPA was then gavaged at 8:30 AM and again at noon. The dyskinesia scores provided in the figures and tables were all obtained during the afternoon L-DOPA dosing period (noon to 4:00 PM). Dyskinesias were scored by a blinded rater from the afternoon recordings for a 1-minute period at 30-minute intervals. The total LID scores provided in the figures represent the average ratings performed on Wednesday and Thursday afternoon of each week for 1–4 weeks, as depicted in the timelines. Dyskinesias were rated on a scale of 0 (no dyskinesias) to 4 (severe dyskinesias) as follows: 1 = subtle dyskinesias that were not sustained (<3 trunk movements in a row), 2 = sustained dyskinesias (≥ 3 trunk movements in a row), 3 = moderate dyskinesias that impaired ability to remain stationary, and 4 = severe dyskinesias that were generalized and incapacitating (Tan et al., 2002; Quik et al., 2007, 2013c).

nAChR Drug Treatments. The nAChR drug treatments involved two sets of MPTP-lesioned monkeys designated set A ($n = 23$) and set B ($n = 16$). Set A monkeys had previously been in studies involving administration of nicotine and/or nAChR drugs, followed by a 1-month washout period (nAChR drug primed). Set B monkeys were nAChR drug-naïve at the start of the current study. We used nAChR drug-primed and nAChR drug-naïve monkeys to assess whether previous nAChR drug treatment modulated the ability of subsequent agonists to reduce LIDs. For both set A (nAChR drug primed) and set B (nAChR drug naïve), there were three experimental groups of monkeys. These included a vehicle-treated group ($n = 6$), a nicotine-treated group ($n = 5$), and an nAChR drug-treated group ($n = 5$ –12).

All monkeys in set A and set B were given 50% diluted orange Gatorade in the drinking water for 1–2 weeks as a control for the nicotine-treated group, since nicotine was administered in Gatorade. Gatorade was included in the drinking water to mask the bitter taste of nicotine [(–)-nicotine, free base; Sigma-Aldrich]. The control and nAChR agonist-treated monkeys were continued on Gatorade for the remainder of the study, whereas the nicotine-treated group received Gatorade plus nicotine. Nicotine was initially given in the Gatorade starting at 50 $\mu\text{g/ml}$ for 2–3 days and was then increased to 150 $\mu\text{g/ml}$ for an additional 3–4 days. The monkeys were maintained at a final concentration of 300 $\mu\text{g/ml}$ nicotine in the drinking water. This dose of oral nicotine was used as our previous studies have shown that it yields monkey plasma nicotine levels of 10–15 ng/ml, which are similar to those in moderate smokers (Quik et al., 2006). Twenty-five milliliters of Gatorade plus nicotine or Gatorade only was also added to the monkey chow.

For the nAChR drug-treated groups in set A and/or set B, the lower doses of varenicline (0.003, 0.01, and 0.03 mg/kg) and TC-8831 (0.01 and 0.03 mg/kg) were given orally in a cracker, twice daily, 30 minutes before the monkeys were gavaged with L-DOPA. Varenicline was obtained from F.I.C. and TC-8831 from Targacept (Winston-Salem, NC; structure published in Quik et al., 2013a). The drugs were applied to the cracker in a 30–40 μl aliquot of water. The monkeys

refused to take the crackers containing the higher doses of varenicline (0.06 and 0.1 mg/kg) and TC-8831 (0.05, 0.1, and 0.3 mg/kg). The drugs were therefore given orally at the same time as the L-DOPA gavage.

Cognitive Testing. The test used to evaluate learning/memory involved the ability to inhibit goal-directed behavior and is thought to reflect a component of spatially delayed working memory in monkeys, which may involve precortical areas (Lyons et al., 2000, 2004). The apparatus used for evaluating cognitive performance was similar to that previously described for use with squirrel monkeys (Lyons et al., 2000, 2004). It consists of a clear Plexiglas box (8 × 8 × 8 cm) with one open side, containing a small piece of marshmallow. The box is locked into place on a metallic platform hooked onto the front of the monkey cage. The test was conducted prior to the morning feeding. Control and MPTP-lesioned monkeys were trained for ~1 month with the box open at various orientations, and were scored for the average time and number of trials required to retrieve the marshmallow piece within a 30-second period (Lyons et al., 2000; Lyons and Schatzberg, 2003). When the monkey performance on these tasks had plateaued to a steady-state level, the effect of nAChR drug administration was tested once weekly (Friday).

Data Analyses. L-DOPA was given on a 5-days-on/2-days-off schedule with parkinsonian ratings performed weekly on Monday and cognitive testing on Friday. Dyskinesia ratings were determined by averaging the scores on Wednesday and Thursday afternoon of each week. Total dyskinesias were determined by calculating area under the curve, and the data were analyzed using two-way analysis of variance (ANOVA) followed by a Bonferroni post-hoc test. Differences in rating scores between groups were analyzed using non-parametric tests (Mann-Whitney test). The data are the median or the mean ± S.E.M. of the number of animals, as indicated in the legends. $P \leq 0.05$ was used for statistical significance.

Results

Varenicline Treatment Reduces LIDs. Varenicline administration was initiated at a very low dose (0.003 mg/kg), as preliminary experiments indicated that vomiting developed with a higher starting dose. Each dose of varenicline (0.003–0.10 mg/kg) was given twice daily, 5 days per week for the number of weeks indicated in the timelines above the data (Fig. 1), with the data representing the mean total LID score (expressed as % vehicle) for the specific dose over the 2–3-week period. Varenicline treatment decreased LIDs at 0.03 mg/kg by ~45%, with a similar decline at the two higher doses (0.06 and 0.10 mg/kg). There was a significant main effect of treatment ($F_{1,80} = 52.08$, $P < 0.001$), dose ($F_{4,80} = 3.86$, $P < 0.01$), and a significant interaction ($F_{4,80} = 3.86$, $P < 0.01$) using two-way ANOVA. Concurrent nicotine treatment in another group of monkeys led to a somewhat greater decline in LIDs, with a significant main effect of treatment ($F_{1,45} = 172.7$, $P < 0.001$), but not dose ($F_{4,45} = 1.83$, $P > 0.05$), and no significant interaction ($F_{4,45} = 1.83$, $P > 0.05$) using two-way ANOVA (Fig. 1).

The afternoon hourly time course of the development of LIDs after L-DOPA gavage is shown in Fig. 2. L-DOPA treatment maximally increased LIDs in vehicle-treated monkeys 30 minutes after gavage, with the response lasting 3.5 hours. Varenicline dosing reduced LIDs over the entire 3.5-hour period. The data shown are for the 0.03 mg/kg dose, with the same pattern for the 0.06 and 0.10 mg/kg doses of varenicline. The effect of nicotine is shown for comparison. It yielded a greater decline in LIDs (Fig. 2), consistent with the total LIDs scores provided in Fig. 1.

Fig. 1. Varenicline treatment reduces LIDs. MPTP-lesioned monkeys were gavaged with L-DOPA twice daily, 5 days per week. Varenicline was given orally twice daily at a 3.5-hour interval at the same time as L-DOPA. The timeline is depicted in the upper panel. The middle panel shows the total dyskinesia scores (expressed as % vehicle) averaged over 2–3 days during the 4-hour period following the afternoon dose of L-DOPA (middle panel). For comparison, the lower panel illustrates the effect of nicotine (300 µg/ml in the drinking water) on LIDs for the same time period as the varenicline data. Values are the mean ± S.E.M. of 5–12 monkeys. Significance of difference of drug treatment from vehicle: *** $P < 0.001$ using two-way ANOVA followed by a Bonferroni post-hoc test.

Experiments were conducted next to determine whether the varenicline-induced reduction in LIDs persisted with drug removal. Five weeks of varenicline washout were required before LIDs returned to the levels seen in vehicle-treated monkeys, with a similar time period required for LIDs to return to vehicle levels with nicotine removal (Table 1).

Varenicline had no significant effect on parkinsonian ratings as compared with the vehicle-treated group (Table 2).

Fig. 2. Varenicline treatment decreases the hourly time course of LIDs. Monkeys were treated as described in the legend of Fig. 1. The data shown are for the afternoon 0.03 mg/kg varenicline dose averaged over several days. The effect of nicotine on LIDs is shown in the lower panel for comparison. The symbols depict the median of 5–12 monkeys. Significance of difference from vehicle using a Mann–Whitney test: *** $P < 0.001$.

These data are consistent with those previously observed with nicotine treatment, which also does not alter parkinsonism (Quik et al., 2007, 2013c). There were no adverse effects of varenicline on fluid intake, body weight, or general behaviors, except for the vomiting mentioned earlier.

TABLE 1

Loss of the nAChR-mediated improvement in LIDs with drug removal. MPTP-lesioned monkeys were gavaged with L-DOPA and given vehicle, nicotine, varenicline, or TC-8831 according to the treatment regimens depicted in Fig. 1 for varenicline and Fig. 3 for TC-8831. Drug removal led to a return of LIDs to vehicle-treated values by 1–6 weeks after washout. Values are the mean $\pm$ S.E.M. of the number of monkeys indicated in parentheses.

nAChR Drug and Time Point	Total LID Scores (% Vehicle)	
	Vehicle	nAChR Drug
Varenicline		
Last week treatment	100 $\pm$ 8.8 (6)	50 $\pm$ 4.9 (12)**
Week 1 washout	100 $\pm$ 4.2 (6)	66 $\pm$ 8.4 (12)*
Week 3 washout	100 $\pm$ 7.5 (6)	70 $\pm$ 8.6 (12)
Week 6 washout	100 $\pm$ 14.9 (6)	90 $\pm$ 4.5 (12)
Nicotine		
Last week treatment	100 $\pm$ 8.8 (6)	22 $\pm$ 2.8 (5)***
Week 1 washout	100 $\pm$ 4.2 (6)	48 $\pm$ 8.0 (5)***
Week 3 washout	100 $\pm$ 7.5 (6)	61 $\pm$ 7.3 (5)**
Week 6 washout	100 $\pm$ 14.9 (6)	92 $\pm$ 9.6 (5)
TC-8831		
Last week treatment	100 $\pm$ 8.5 (6)	45 $\pm$ 11 (6)**
Week 1 washout	100 $\pm$ 6.2 (6)	102 $\pm$ 4.1 (6)

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ using a t test (significance of difference from vehicle).

TABLE 2

nAChR agonist treatment does not affect parkinsonism

Monkeys were rated for parkinsonism once weekly throughout the study, starting 3–4 weeks after the last MPTP injection. The parkinsonian ratings shown were obtained in the same week as the data provided in Fig. 2 for varenicline and Fig. 4 for TC-8831, with similar results throughout the study. Values are the mean $\pm$ S.E.M. of the number of monkeys indicated in parentheses.

Study	Parkinsonian Scores		
	Vehicle	Nicotine	nAChR Agonist
Set A: varenicline	4.7 $\pm$ 0.4 (6)	3.6 $\pm$ 0.2 (5)	4.0 $\pm$ 0.3 (12)
Set A: TC-8831	6.0 $\pm$ 1.3 (6)	5.2 $\pm$ 0.4 (5)	6.6 $\pm$ 0.8 (5)
Set B: TC-8831	5.3 $\pm$ 1.1 (6)	5.0 $\pm$ 0.4 (5)	5.8 $\pm$ 1.0 (5)

TC-8831 Treatment Reduces LIDs. Two sets of MPTP-lesioned monkeys were used to investigate the effect of TC-8831 on LIDs. Set A monkeys (nAChR drug primed, $n = 17$) had previously been treated with nicotine and varenicline, followed by a 10-week washout period. Set B monkeys ($n = 16$) were nAChR drug naïve. Each set of monkeys was subdivided into three experimental groups as follows: vehicle-treated ($n = 6$), nicotine-treated ($n = 5$), and TC-8831-treated ($n = 5$ –6). All TC-8831 doses were given twice daily, 5 days per week for the number of weeks indicated in the timelines above the data, with the data representing the mean total LID score (expressed as % vehicle) for the specific dose over the designated number of weeks. TC-8831 administration was started at 0.01 mg/kg; this dose had no effect on total LID scores averaged during the week (Fig. 3). The TC-8831 dose was then increased to 0.03 mg/kg for 2 weeks (Fig. 3). Significant declines ($\sim 30\%$) were observed in LIDs in both sets of monkeys (Fig. 3). In set A, a maximal decline in LIDs was obtained with the 0.10 mg/kg dose, with similar results with 0.30 mg/kg (each dose given for 2 weeks). There was a significant main effect of treatment ($F_{1,40} = 24.41$, $P < 0.001$), but not dose ($F_{3,40} = 1.41$, $P > 0.05$), and no significant interaction ($F_{3,40} = 1.41$, $P > 0.05$) using two-way ANOVA. A decline was also observed in the set B monkeys administered 0.10 mg/kg TC-8831 for 4 weeks, with a significant main effect of treatment ($F_{1,27} = 21.26$, $P < 0.001$), dose ($F_{2,27} = 3.99$, $P < 0.01$), and a significant interaction ($F_{2,27} = 3.99$, $P < 0.01$) using two-way ANOVA. However, the 0.30 mg/kg dose induced vomiting in the set B monkeys; the data were therefore not included, as the emesis could interfere with uptake of both L-DOPA and TC-8831 and, thus, LIDs.

Nicotine treatment led to 50%–80% declines in LIDs in set A (Fig. 3), with a significant main effect of treatment ($F_{1,36} = 146.7$, $P < 0.001$), but not dose ($F_{3,36} = 2.4$, $P > 0.05$), and no significant interaction ($F_{3,36} = 2.4$, $P > 0.05$) using two-way ANOVA. These data are consistent with previous results with long-term nicotine dosing (Quik et al., 2013c,d). These earlier studies also showed that up to 8 weeks of nicotine treatment is required for a maximal decline in LIDs (Quik et al., 2013c,d). The data in the right-hand panels in Fig. 3 are consistent with these previous studies, with a smaller nicotine-mediated decline in LIDs in the set B monkeys that had not previously received nicotine (Quik et al., 2013c,d). In set B, there was a significant main effect of treatment ($F_{1,27} = 8.172$, $P < 0.01$), but not dose ($F_{2,27} = 2.49$, $P > 0.05$), and no significant interaction ($F_{2,27} = 2.49$, $P > 0.05$) using two-way ANOVA.

The afternoon hourly time courses demonstrate that TC-8831 reduced LIDs throughout the course of L-DOPA

Fig. 3. TC-8831 treatment decreases LIDs. The effect of TC-8831 was tested in a set of MPTP-lesioned monkeys previously exposed to nicotine and nAChR drugs (set A), as well as in a set of nAChR drug-naïve MPTP-lesioned monkeys (set B). The monkeys were gavaged with L-DOPA twice daily, 5 days per week. TC-8831 (0.01–0.30 mg/kg) was given orally twice daily at a 3.5-hour interval at the same time as L-DOPA. The timeline is depicted in the upper panel. The middle panel shows the total dyskinesia scores (expressed as % vehicle) averaged over 2–3 days during the 4-hour period following the afternoon dose of L-DOPA. For comparison, the lower panel illustrates the effect of nicotine (300 µg/ml in the drinking water) on LIDs for the same time period as the TC-8831 data. Values are the mean ± S.E.M. of 5–6 monkeys. Significance of difference of drug treatment from vehicle: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ using two-way ANOVA followed by a Bonferroni post-hoc test.

administration in both sets of monkeys (Fig. 4). The effect of TC-8831 was thus similar to that of nicotine, shown for comparison (Fig. 4). The data provided are for the 0.10 mg/kg dose (depicted in Fig. 3), with results similar to the other doses of TC-8831 tested.

After the initial dose-response studies depicted in Figs. 3 and 4, we repeated the 0.10 and/or 0.30 mg/kg doses for several weeks each. However, vomiting was observed and the data were therefore not used. The animals were then placed on a drug washout, with LIDs returning to the levels seen in vehicle-treated monkeys after 1 week (Table 1).

TC-8831 was subsequently retested for its effect on LIDs at the lowest doses initially used (Fig. 5). In the repeat TC-8831 dosing regimen, the maximal decline in LIDs was somewhat greater (50–70%) than in the initial study shown in Fig. 3. In set A, there was a significant main effect of treatment ($F_{1,30} = 19.92$, $P < 0.001$), but not dose ($F_{2,30} = 2.51$, $P > 0.05$), and no significant interaction ($F_{2,30} = 2.51$, $P > 0.05$) using two-way

ANOVA. In set B, there was a significant main effect of treatment ($F_{1,26} = 104.9$, $P < 0.001$), but not dose ($F_{2,26} = 0.57$, $P > 0.05$), and no significant interaction ($F_{2,26} = 0.57$, $P > 0.05$) using two-way ANOVA. Interestingly, the decline in LIDs was similar to that of nicotine. In set A, there was a significant main effect of treatment ($F_{1,27} = 87.70$, $P < 0.001$), but not dose ($F_{2,27} = 0.086$, $P > 0.05$), and no significant interaction ($F_{2,27} = 0.086$, $P > 0.05$) using two-way ANOVA. In set B, there was a significant main effect of treatment ($F_{1,27} = 62.07$, $P < 0.001$), but not dose ($F_{2,27} = 0.069$, $P > 0.05$), and no significant interaction ($F_{2,27} = 0.069$, $P > 0.05$) using two-way ANOVA. There was no vomiting with the lower doses (0.01 and 0.03 mg/kg) of TC-8831. However, intermittent vomiting developed with the 0.05 mg/kg dose; higher TC-8831 doses were therefore not tested. The afternoon hourly time course of the development of LIDs shows that both TC-8831 and nicotine reduced LIDs with a similar time course (Fig. 6).

Fig. 4. TC-8831 treatment decreases the hourly time course of LIDs. TC-8831 was tested in a set of MPTP-lesioned monkeys previously exposed to nicotine and nAChR drugs (set A), as well as in a set of nAChR drug-naïve MPTP-lesioned monkeys (set B). Drug treatments were as described in the legend of Fig. 3, with the data shown for the afternoon 0.10 mg/kg TC-8831 dose averaged over several days. The effect of nicotine on LIDs is shown in the lower panels for comparison. The symbols depict the median of 5–6 monkeys. Significance of difference from vehicle using a Mann-Whitney test: ** $P < 0.01$; *** $P < 0.001$.

TC-8831 readministration did not affect parkinsonism, similar to the results obtained with the initial dosing regimen (Table 2). TC-8831 also had no appreciable effect on a cognitive task designed for squirrel monkeys (Lyons et al., 2000; Lyons and Schatzberg, 2003). The data in Table 3 show no change in the average time and number of trials required for the monkeys to retrieve a marshmallow piece. Since parkinsonism was only mild to moderate, performance in the cognitive task was not affected by motor impairment. There were no adverse effects of TC-8831 on fluid intake, body weight, or general behavior, except for the vomiting at higher drug doses, as noted previously.

Discussion

The present results show that two nAChR agonists with distinct pharmacological profiles reduced LIDs (30–60%) in parkinsonian monkeys. We selected the nAChR agonist varenicline because it is approved by the U.S. Food and Drug Administration and thus offered the benefit that it could be extended to the clinic with minimal delay. TC-8831 has the advantage that it is a CNS-selective nAChR agonist at $\alpha 4\beta 2^*$ and $\alpha 6\beta 2^*$ nAChRs, and thus may be associated with fewer peripheral side effects. Notably, there was no tolerance to the effect of either drug to reduce LIDs for the entire length of the study (3–4 months). Moreover, the drugs did not worsen parkinsonism or cognition. These data suggest that varenicline and TC-8831 may be useful for the treatment of LIDs in Parkinson's disease patients.

The ability of nAChR agonists to reduce LIDs supports the idea that nicotine exerts its antidyskinetic effect via an interaction at nAChRs. As mentioned earlier, there are multiple nAChRs throughout the body, with the predominant populations in the striatum being the $\alpha 4\beta 2^*$ and $\alpha 6\beta 2^*$ nAChR subtypes (Gotti et al., 2009; Millar and Gotti, 2009;

Quirk and Wonnacott, 2011). Although initial studies suggested that varenicline was selective for $\alpha 4\beta 2^*$ nAChRs, subsequent work showed that it also interacted with $\alpha 6\beta 2^*$, $\alpha 3\beta 4^*$, and $\alpha 7$ nAChRs (Coe et al., 2005; Mihalak et al., 2006; Grady et al., 2010; Ween et al., 2010; Chatterjee et al., 2011; Bordia et al., 2012). Varenicline's biological effects may thus be more similar to those of nicotine, which also stimulates multiple nAChR populations. With respect to the clinical relevance of the current dosing regimen, the dose of varenicline prescribed for smoking cessation is 1 mg twice daily. The maximal effective dose of varenicline in our studies was 0.03 mg/kg twice daily, which is similar to the dose used for smoking cessation on a per-weight basis. In humans, varenicline is associated with nausea and vomiting in ~25% of users (Cahill et al., 2011; Swan et al., 2012). However, there were no issues with vomiting in our studies when varenicline treatment was initiated at a low dose and incrementally increased in half-log intervals, suggesting it may be useful for the treatment of LIDs. Notably, a recent clinical trial showed that varenicline also improved gait and stance in patients with spinocerebellar ataxia and was fairly well tolerated (Zesiewicz et al., 2012).

Our data with TC-8831 show that a drug that interacts specifically with CNS $\alpha 4\beta 2^*$ and $\alpha 6\beta 2^*$ nAChRs, those most densely expressed in the striatum, also reduced LIDs. These data are consistent with previous work showing that TC-8831 attenuated LIDs in MPTP-lesioned macaques (Johnston et al., 2013). Our initial dose-response curves are similar to those of Johnston and coworkers, indicating that the effect of the nAChR agonist TC-8831 is comparable in different monkey species. However, there appeared to be a U-shaped dose-response curve in the macaque, suggesting tolerance with time and/or increasing dose (Johnston et al., 2013). The present repeat-dosing regimen clearly shows that no tolerance developed with increased TC-8831 administration. In

Fig. 5. No tolerance to the TC-8831-mediated decline in LIDs with repeated dosing. TC-8831 administration was retested after a 2–3-week washout period in set A (nAChR drug primed) and set B (nAChR drug naïve). The monkeys were gavaged with L-DOPA twice daily, 5 days per week. TC-8831 (0.01–0.05 mg/kg) was given orally twice daily at a 3.5-hour interval at the same time as L-DOPA. The timeline is depicted in the upper panel. The middle panel shows the total dyskinesia scores (expressed as % vehicle) averaged over 2–3 days during the 4-hour period following the afternoon dose of L-DOPA. For comparison, the lower panel illustrates the effect of nicotine (300 µg/ml in the drinking water) on LIDs for the same time period as the TC-8831 data. Values are the mean $\pm$ S.E.M. of 5–6 monkeys. Significance of difference of drug treatment from vehicle: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ using two-way ANOVA followed by a Bonferroni post-hoc test.

fact, a somewhat greater decline in LIDs was observed with a repeat TC-8831 regimen, such that the reduction in LIDs approached that obtained with nicotine. These results suggest that stimulation of $\alpha 4\beta 2^*$ and/or $\alpha 6\beta 2^*$ nAChRs may be sufficient to reduce LIDs.

With respect to the issue of tolerance, the antidyskinetic effect of both varenicline and TC-8831 persisted over the entire 3–4 months of the study. These data are similar to those previously obtained with nicotine, which also consistently reduced LIDs over ~6 months with no indication of a diminished response (Quik et al., 2013d).

Our data further show that previous nAChR drug treatment does not impact the effect of subsequent dosing with other nAChR agonists on LIDs—that is, there is no sensitization or desensitization among the agonists tested, at least after a washout period (10 weeks). By contrast, prolonged use appeared to enhance drug effectiveness in some cases, as nicotine best reduced LIDs after 2 or 3 months of treatment.

In addition, TC-8831 treatment led to a greater decline in LIDs with repeated dosing. However, with the repeat TC-8831 dosing regimen, the monkeys were also more sensitive to vomiting, which now occurred at lower doses.

With respect to mechanism of action, nAChR agonist may improve LIDs in parkinsonian animals by continuously desensitizing or inactivating nAChR-mediated function. Our recent studies show that long-term nicotine treatment decreased dopamine release in parkinsonian rats (Bordia et al., 2013). Thus, nicotine treatment may dampen the aberrant dopamine release which is thought to underlie the development of LIDs (Cenci, 2007; Lindgren et al., 2010; Carta and Bezard, 2011; Fisone and Bezard, 2011). Support for a role for desensitization stems from studies showing that both nAChR agonists and antagonists reduced LIDs to a similar extent in parkinsonian rats (Bordia et al., 2010). In addition, intermittent nicotine exposure via injection or continuous treatment via a slow-release minipump that promotes receptor

Fig. 6. Hourly time course showing no tolerance to the TC-8831-mediated decline in LIDs with continued dosing. TC-8831 was retested in a set of MPTP-lesioned monkeys previously exposed to nicotine and nAChR drugs (set A), as well as in a set of nAChR drug-naïve MPTP-lesioned monkeys (set B). Drug treatments were as described in the legend of Fig. 5, with the data shown for the afternoon 0.03 mg/kg TC-8831 dose averaged over several days. The effect of nicotine on LIDs is shown in the lower panels for comparison. The symbol depicts the median of 5–6 monkeys. Significance of difference from vehicle using a Mann–Whitney test: ***P* < 0.01; ****P* < 0.001.

desensitization both reduce LIDs to a similar extent (Bordia et al., 2008, 2010). The idea that nicotine affects LIDs via desensitization is consistent with its effects in depression, addiction, and pain (Picciotto et al., 2008; Buccafusco et al., 2009; Dopico and Lovinger, 2009) (Mineur and Picciotto, 2010; Zhang et al., 2012).

An extensive literature indicates that nicotine and nAChR drugs may enhance cognition in experimental animal models (Terry and Decker, 2011; Gao et al., 2012). The current data show that varenicline and TC-8831 did not influence performance on a working memory task in MPTP-lesioned monkeys. This finding provides indirect support for the contention that nAChR agonists do not cause sedation.

Parkinsonism was not worsened with either varenicline or TC-8831, in agreement with our previous results showing no effect of nicotine on motor function in parkinsonian animal models (Quik et al., 2007, 2013a,c). This finding is consistent with the results of clinical reports and trials in Parkinson’s disease patients, which also report no consistent improvement in motor tasks. Although the open-label studies generally demonstrated a positive effect of nicotine on motor symptoms (Marshall and Schnieden, 1966; Ishikawa and Miyatake, 1993; Kelton et al., 2000; Mitsuoka et al., 2002; Lemay et al., 2004; Hanagasi et al., 2007; Villafane et al.,

2007), there was very little clinical efficacy of nicotine in the double-blinded trials, which also included significantly larger groups of patients (Fagerstrom et al., 1994; Clemens et al., 1995; Ebersbach et al., 1999; Vieregge et al., 2001; Shoulson, 2006). Although the variability of outcomes in the clinical studies may be due to differences in nicotine dosing, duration of treatment, outcome measure evaluated, small cohorts, and/or clinical stage, the nature of the type of trial, that is, open-label versus double-blinded, may be the important variable. The finding that nicotine and nAChR agonists improve LIDs with no apparent effect on parkinsonism is consistent with preclinical studies, suggesting that these two behaviors may be differentially regulated by the direct and indirect pathways. Activation of the direct pathway may be more closely associated with LIDs, whereas parkinsonism may be linked to activation of the indirect pathway (Santini et al., 2008; Kravitz et al., 2010; Huot et al., 2013).

In summary, the present results show that nAChR agonists reduce LIDs in a nonhuman primate parkinsonian model. These data provide proof of concept for the idea that drugs that interact with CNS nAChRs may be of value as antidyskinetic agents in Parkinson’s disease. Moreover, both drugs were effective following oral administration, a mode of administration that readily extends to human use.

TABLE 3
TC-8831 and nicotine treatment did not modulate cognitive performance
MPTP-lesioned monkeys were gavaged with L-DOPA and given vehicle, nicotine, or TC-8831 according to the treatment regimens depicted in Fig. 3 for TC-8831. The data shown were obtained in the same week as those provided in Fig. 4, with similar results throughout the study. Values are the mean ± S.E.M. of the number of monkeys indicated in parentheses.

Set	Time to Retrieve Treat			Number of Trials to Retrieve Treat		
	Vehicle	Nicotine	TC-8831	Vehicle	Nicotine	TC-8831
	sec	sec	sec			
A	8.3 ± 2.2 (6)	8.0 ± 1.6 (5)	8.0 ± 2.0 (6)	6.7 ± 1.4 (6)	6.6 ± 1.6 (5)	7.7 ± 2.2 (6)
B	13.2 ± 3.4 (6)	13.8 ± 4.1 (5)	12.0 ± 3.0 (5)	14.6 ± 3.2 (6)	13.7 ± 2.6 (5)	13.6 ± 2.7 (5)

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Authorship Contributions

Participated in research design: Quik, Zhang, Mallela, Letchworth.
Conducted experiments: Zhang, Mallela, Sohn, Quik.
Contributed new agents or analytic tools: Carroll, Bencherif, Letchworth.
Performed data analysis: Zhang, Mallela, Sohn, Quik.
Wrote or contributed to the writing of the manuscript: Quik, Zhang, Mallela.

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