Continuous and Intermittent Nicotine Treatment Reduces L-3,4-Dihydroxyphenylalanine (L-DOPA)-Induced Dyskinesias in a Rat Model of Parkinson’s Disease

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ABSTRACT

The development of abnormal involuntary movements (AIMs) or dyskinesias is a serious complication of L-DOPA [L-3,4-dihydroxyphenylalanine] therapy for Parkinson’s disease. Our previous work had shown that intermittent nicotine dosing reduced L-DOPA-induced dyskinetic-like movements in nonhuman primates. A readily available nicotine formulation is the nicotine patch, which provides a constant source of nicotine. However, constant nicotine administration more readily desensitizes nicotinic receptors, to possibly yield alternate behavioral outcomes. Therefore, we investigated whether constant nicotine administration reduced L-DOPA-induced AIMs in a rat parkinsonian model, with results compared with those with intermittent nicotine dosing. Rats with a unilateral 6-hydroxydopamine (6-OHDA) lesion were exposed to either intermittent (drinking water) or constant (minipump) nicotine for 2 weeks at doses that yielded plasma levels of the nicotine metabolite cotinine similar to those in smokers. The rats were next treated with L-DOPA/benserazide (8 or 12 mg/kg/15 mg/kg) for ≥3 weeks to allow for the development of AIMs, with nicotine treatment continued. Both modes of nicotine administration resulted in ≥50% decline in L-DOPA-induced AIMs. Nicotine treatment also significantly reduced AIMs in L-DOPA-primed rats using either dosing regimen, whereas nicotine removal led to an increase in AIMs. There was no effect of nicotine on various measures of motor performance in 6-OHDA-lesioned rats. In summary, nicotine provided either via the drinking water or minipump reduced L-DOPA-induced AIMs in a rat model of Parkinson’s disease. These results suggest that either intermittent or constant nicotine treatment may be useful in the treatment of L-DOPA-induced dyskinesias in patients with Parkinson’s disease.

Dyskinesias are a complication of L-DOPA treatment that eventually develop in the majority of patients with Parkinson’s disease (Fabbriani et al., 2007; Singh et al., 2007; Santini et al., 2008). These abnormal movements can be quite debilitating and represent a major drawback to continued L-DOPA therapy. A variety of drugs targeting various neurotransmitter systems in the basal ganglia have been reported to exert beneficial effects against dyskinetic-like movements in parkinsonian animal models, including the glutamate, adenosine, noradrenaline, 5-hydroxytryptamine, cannabinoid, and opioid systems (Brotchie, 2005; Fox et al., 2006; Fabbriani et al., 2007). However, management of L-DOPA-induced side effects in patients with Parkinson’s disease continues to represent a serious therapeutic challenge.

Therefore, there is a continual search for new approaches and alternative agents that may reduce this side effect associated with L-DOPA therapy. One drug that has recently been shown to attenuate L-DOPA-induced dyskinetic-like movements in nonhuman primates is nicotine (Quik et al., 2007b). Nicotine may exert this effect by acting on nicotinic acetylcholine receptors, which are present on dopaminergic terminals in the striatum and involved in regulating dopamine release (Gotti et al., 2006; Grady et al., 2007; Quik et al., 2007a). Nicotine reduces L-DOPA-induced dyskinetic-like movements in both L-DOPA naive and L-DOPA-primed monkeys when administered via the drinking water. This could be classified as an intermittent mode of treatment because animals drink sporadically during the course of the day. Another form of nicotine dosing is continuous administration, which offers the advantage that it is readily available for humans in the form of the nicotine patch. However, these two modes of nicotine exposure are quite distinct because there is a continual, constant release of nicotine from the patch with no peak and trough nicotine levels as would occur.
with intake via the drinking water. The type of delivery is of relevance to the current study because constant exposure to nicotine is well known to result in nicotinic receptor desensitization, with a consequent loss of receptor-mediated function (Gniatullin et al., 2005; Wang and Sun, 2005; Picciotto et al., 2007). On the other hand, desensitization is also associated with changes in nicotinic receptor expression, which may influence nicotinic receptor-mediated behaviors.

The present studies were done to determine whether constant nicotine exposure, which probably induces a greater degree of receptor desensitization than intermittent administration via the drinking water, reduces the incidence of L-DOPA-induced AIMS. For these studies, we used a well-characterized and validated rodent model, L-DOPA-treated rats with a unilateral 6-hydroxydopamine (6-OHDA) lesion of the nigrostriatal pathway (Cenci et al., 1998, 2002; Lundblad et al., 2002; Cenci and Lindgren, 2007; Dekundy et al., 2007). Nicotine was administered via minipump to provide a constant source of drug, as well as in the drinking water to provide an intermittent dosing regimen. The results show that both forms of nicotine exposure result in significant declines in abnormal involuntary movements (AIMs) in L-DOPA-naive and L-DOPA-primed rats.

**Materials and Methods**

**Animals.** Experiments were performed using male Sprague-Dawley rats (initial weight ~250 g) that were purchased from Charles River Laboratories, Inc. (Wilmington, DE). They were housed two per cage under a 12 to 12-h light/dark cycle from 7:00 AM to 7:00 PM in a temperature-controlled room with free access to food and water. Three to 4 days after arrival, the rats were unilaterally lesioned with 6-OHDA (Sigma-Aldrich, St. Louis, MO) as described previously (Cenci et al., 1998, 2002). During the lesioning procedure, the rats were maintained under isoflurane anesthesia (2%). They were placed in a Kopf stereotaxic instrument (David Kopf Instruments, Tujunga, CA), and burr holes were drilled through the skull at the following coordinates relative to the bregma and dural surface: 1) anteroposterior, −4.4; lateral, 1.2; ventral, 7.8; tooth bar at −2.4; and 2) anteroposterior, −4.0; lateral, 0.75; ventral, 8.0; tooth bar at +3.4 (Cenci et al., 1998, 2002). 6-OHDA was dissolved in 0.02% ascorbic acid/saline at a concentration of 5 µg/µl. Two microliters of aliquots was stereotaxically injected at each of these sites for a total of 12 µg into the right-ascending, dopamine-fiber bundle. Infusion of 6-OHDA into the target area was over a 2-min period, with the cannula maintained at the site of injection for an additional 2 min. After surgery, rats were administered buprenorphine (0.02 mg/kg s.c.) for postoperative pain. All procedures conformed to the Institute of Laboratory Animal Resources (1996) and were approved by the Institutional Animal Care and Use Committee.

**Testing for Rotational Behavior.** At different time points after lesioning, rats were tested for drug-induced rotational behavior in an automated behavioral measurement apparatus (ROTOMAX; Accu-Scan Instruments, Inc., Columbus, OH). Each rat was placed in a cylindrical glass chamber for 30 min for acclimatization, after which 4.0 mg/kg amphetamine (Sigma-Aldrich) was administered intraperitoneally. Amphetamine-induced rotational behavior was monitored for 90 to 120 min, with rats making at least 100 ipsilateral turns used for further study.

**Nicotine Treatment via the Drinking Water.** In the initial series of experiments, nicotine (freebase; Sigma-Aldrich) was administered to lesioned rats via the drinking water as outlined in Fig. 1

**Fig. 1.** Intermittent nicotine treatment reduces L-DOPA-induced AIMS. Treatment schedule (top panel) depicting the schedule of nicotine administration in drinking water, L-DOPA dosing, and behavioral testing. 6-OHDA-lesioned rats were given drinking water containing 1% saccharin for 1 week. Half of the rats (n = 10) were continued on saccharin-containing water, whereas the remainder (n = 9) received the same solution also containing nicotine. Nicotine was given at a dose of 25 µg/ml for 3 days and then switched to a final maintenance dose of 50 µg/ml. Three weeks later, the rats were given L-DOPA (8 mg/kg i.p.) once daily with the nicotine continued. After 5 weeks of treatment with 8 mg/kg L-DOPA, the nicotine dose was decreased to 25 µg/ml and the L-DOPA was maintained. After 4 weeks on this latter dose of nicotine, treatment with L-DOPA was increased to 12 mg/kg for an additional 5 weeks, with the nicotine continued. The rats were evaluated for axial, oral, and forelimb AIMS several times (AIMs I, II, and III) throughout the L-DOPA treatment by two raters, one blinded to the treatment status of the animals (bottom panels). The total AIMS score (sum of the axial, oral, and forelimb components) is provided. AIMS were first assessed over a 20-min baseline period (time = 0) and then for an additional 3-h period after L-DOPA treatment, as described under Materials and Methods. There was a significant main effect of nicotine (p < 0.05) on L-DOPA-induced AIMS during sessions II and III using ANOVA, with a trend for a decrease in AIMS I. Each symbol is the mean ± S.E.M. of 9 to 10 rats.
to determine whether this mode of administration reduced L-DOPA-induced involuntary movements in parkinsonian rats, as it had in monkeys (Quik et al., 2007b). Five weeks after 6-OHDA lesioning, rats were given water containing 1% saccharin (Sigma-Aldrich) to mask the bitter taste of nicotine. Two to 3 days later, nicotine was added to the saccharin-containing drinking water, pH 7.0, of the treated group. Nicotine was initially administered at a concentration of 25 μg/ml nicotine for 2 days. This was increased to 50 μg/ml nicotine, and the rats were maintained at this dose for several weeks. Rats with nicotine in the drinking water drank less than animals receiving only water (33 ± 1 versus 45 ± 6 ml/day), whereas animals on saccharin drank more than animals on water (50 ± 3 ml/day), in agreement with previous studies in mice (Lai et al., 2005). All of the rats seemed healthy, although the saccharin-treated rats weighed significantly more (608 ± 20 g, n = 10) than the nicotine-treated animals (529 ± 14 g, n = 9) over the ~8-month period of the study (p < 0.01).

Three weeks after the start of nicotine dosing, the rats received single daily intraperitoneal injections of 8 mg/kg L-DOPA methyl ester (Sigma-Aldrich) for 5 weeks, with the nicotine continued at 50 μg/ml (Fig. 1, top). L-DOPA was administered together with 15 mg/kg benserazide (Sigma-Aldrich) to inhibit peripheral aromatic L-amino acid decarboxylase and optimize delivery of L-DOPA to the brain. After 5 weeks of L-DOPA treatment, the dose of nicotine was decreased to 25 μg/ml because the rats receiving both 50 μg/ml nicotine and L-DOPA seemed nervous and irritable. These behaviors disappeared with the reduction in nicotine dose. After ~4 weeks on 25 μg/ml nicotine, the L-DOPA dose was increased to 12 mg/kg for an additional 6 weeks, with the nicotine continued at 25 μg/ml. This was done to determine the effectiveness of nicotine with increased L-DOPA dosing.

Nicotine Treatment via Minipump. In a separate series of experiments (Fig. 4, top), nicotine was systemically infused via osmotic minipumps (Alzet model 2004; Durect Corporation, Cupertino, CA), which delivered nicotine at a rate of 6 μl/day for 28 days. Pumps were prefilled with either sterilized water or nicotine base in water, pH 7.0, to provide a dose of 2 mg/kg/day. The minipumps were subcutaneously implanted along the dorsal aspect of the neck between the shoulder blades, according to the manufacturer’s instructions, under isoflurane anesthesia. After implantation, rats were administered buprenorphine (0.02 mg/kg s.c.) for postoperative pain. Two weeks after nicotine treatment was initiated, the rats were given single daily intraperitoneal injections of 8 mg/kg L-DOPA methyl ester plus 15 mg/kg benserazide for 4 weeks followed by several more weeks of L-DOPA at 12 mg/kg L-DOPA methyl ester plus 15 mg/kg benserazide (Fig. 4, top). Four weeks after implantation, the minipumps were removed, and new pumps releasing nicotine for 6 weeks (Alzet model 2006; Durect Corporation) were subcutaneously implanted at the same site.

Measurement of L-DOPA-Induced AIMS. L-DOPA-induced AIMS were determined 3 weeks after daily L-DOPA dosing, as well as at various other time points as indicated in the dosing schedules. Behavioral testing was done in the morning between 9:00 and 10:00 AM. Three different AIMS subtypes were measured including the following: 1) axial dystonia, contralateral twisted posturing of the neck and upper body; 2) abnormal orolingual movements, stereotyped jaw movements and contralateral tongue protrusion; and 3) abnormal forelimb movements, repetitive rhythmic jerks or dystonic posturing of the contralateral forelimb and/or grabbing movements of the contralateral paw (Cenci et al., 1998, 2002; Carta et al., 2006). Rats were scored on a scale from 0 to 4 for each of these three AIMS subtypes as follows: 1 = occasional; 2 = frequent; 3 = continuous but interrupted by sensory distraction; and 4 = continuous, severe, not interrupted by sensory distraction. Animals were evaluated for AIMS for an initial 20-min baseline period as well as for 3 h after L-DOPA injection. Two raters, one blinded to treatment, performed the AIMS ratings, with the ratings representing the average scores of the two observers. There was an excellent correlation in AIMS scores between the two observers (r = 0.99, p < 0.001). Assessment of the different subtypes of AIMS was done over 20-min sessions, thus yielding a total of 9 sessions of testing per animals. The maximal possible score for each animal was thus 108 (maximal score per session = 12; number of sessions over 3 h = 9).

Limb Use Asymmetry Test. The forelimb asymmetry (cylinder) test was used as an index of behavioral function after unilateral nigrostriatal damage, with explorative activity analyzed as described previously (Schallert et al., 2000; Tillerson et al., 2002). Animals were placed in a transparent cylinder (20-cm diameter × 30-cm height) or in a transparent cage and evaluated over a 5-min period. A mirror was placed behind the cylinder/cage to allow the raters to view forelimb movements when the rat was turned away from the rater. Wall exploration and landing scores were determined separately and expressed in the following terms: 1) the percentage of use of the nonimpaired forelimb (ipsilateral to the lesion) compared with the total number of limb use movements; 2) the percentage of use of the impaired forelimb (contralateral to the lesion) compared with the total number of limb use movements; 3) simultaneous use of both the left and right forelimb for wall contact during a full rear and for lateral movements along the wall; and 4) simultaneous use of both the left and right forelimb for landing after a rear. Two raters, one blinded to the treatment status of the rats, performed the ratings. Correlation analyses yielded a high inter-rater reliability (r = 0.99).

Plasma Cotinine Measurement. The nicotine metabolite cotinine was measured as an index of nicotine intake because it is one of the primary metabolites of nicotine with a relatively long half-life (~20 h) (Kinn et al., 2007). Cotinine levels provide a fairly accurate measure of nicotine intake, as previously shown (Lai et al., 2005; Quik et al., 2006; Kinn et al., 2007). Blood samples were collected from the lateral saphenous vein 1 to 2 weeks after initiation of nicotine treatment via either the drinking water or minipump. Plasma was prepared and cotinine was assayed using an ELISA kit (OraSure Technologies, Bethlehem, PA) according to the manufacturer’s instructions. A standard curve ranging from 1 to 75 ng/ml cotinine was done with every assay.

[125I]RTI-121 Autoradiography. Rats were killed by CO2 exposure followed by decapitation. The brains were rapidly removed, quick frozen in isopentane on dry ice, and stored at −80°C. Frozen brains were sectioned (8 μm) at −20°C using a cryostat, and the sections were thaw mounted onto poly-l-lysine-coated slides, dried, and stored at −80°C. Dopamine transporter measurements were performed using [125I]RTI-121 (2200 Ci/mmol; PerkinElmer Life and Analytical Sciences, Waltham, MA) as described previously (Quik et al., 2006). Thawed sections were preincubated at room temperature for 2 × 15 min in 50 mM Tris-HCl, pH 7.4, 120 mM NaCl, and 5 mM KCl (buffer), followed by incubation (2 h) in buffer also containing 0.025% bovine serum albumin, 1 μM fluoxetine, and 100 pM [125I]RTI-121. Sections were washed four times for 15 min each in ice-cold buffer and then once in ice-cold water. They were air dried and exposed to Kodak MR film (PerkinElmer Life and Analytical Sciences) for 2 days with [125I]-microscale standards (GE Healthcare, Little Chalfont, Buckinghamshire, UK). Binding in the presence of nomifensine (100 μM) was used to evaluate the blank.

Data Analyses. All analyses were done using GraphPad Prism (GraphPad Software, Inc., San Diego, CA). Values are the mean ± S.E.M. of the indicated number of rats and represent data from one to two separate testing periods. Differences in rating scores between groups were analyzed using nonparametric tests (Mann-Whitney, Mann-Whitney U test or Wilcoxon test for paired data). For the time-course studies, repeated measures analysis of variance (ANOVA) followed by Bonferroni’s multiple comparison test were used. A level of 0.05 was considered significant.

Results

Nicotine Administration via the Drinking Water

Decline in L-DOPA-Induced AIMS in Lesioned Rats with Intermittent Nicotine Treatment. After ≤3 weeks of nicotine treatment via the drinking water, rats showed a reduction in L-DOPA-Induced AIMS, as indicated by the decline in the percentage of use of the nonimpaired forelimb (ipsilateral to the lesion) compared with the total number of limb use movements (Fig. 2A). This effect was significant at all nicotine doses tested (Fig. 2B). The percentage of use of the impaired forelimb (contralateral to the lesion) compared with the total number of limb use movements also showed a decline with nicotine treatment (Fig. 2C). However, this effect was not statistically significant. Simultaneous use of both the left and right forelimb for wall contact decreased with nicotine treatment, but this effect was not statistically significant (Fig. 2D). Simultaneous use of both the left and right forelimb for landing after a rear also decreased with nicotine treatment, but this effect was not statistically significant (Fig. 2E). These results suggest that nicotine treatment via the drinking water decreased L-DOPA-Induced AIMS in lesioned rats.
of L-DOPA administration to saccharin-treated rats, significant AIMs developed that were maximal by 20 to 80 min after injection and then gradually declined (Fig. 1, bottom panels). In rats pretreated with 50 µg/ml nicotine in the drinking water, there was a trend for a decline in total L-DOPA-induced AIMs compared with rats not receiving nicotine (AIMs I; Figs. 1 and 2). This seemed to be due to a reduction in forelimb AIMs (p < 0.05) as well as oral (p < 0.07) AIMs (AIMs I; Fig. 2).

The 50 µg/ml nicotine dose led to relatively high plasma cotinine levels (Table 1). For this reason, and also because of increased nervousness and irritability of the rats with L-DOPA treatment, the nicotine was reduced to 25 µg/ml. This dose yielded rat plasma cotinine levels within the range in moderate smokers (Table 1). In addition, the increased nervousness and irritability disappeared. The 25 µg/ml nicotine dose resulted in a reduction in L-DOPA-induced AIMs, with a significant main effect of treatment (p < 0.05) over the 3-h time period after L-DOPA treatment (AIMs II; Fig. 1). Evaluation of the individual AIM subtypes showed that nicotine significantly decreased both axial and forelimb AIMs with a trend for improvement in oral AIMs (AIMs II; Fig. 2). The significant decline in AIMs with the 25 µg/ml compared with the 50 µg/ml nicotine dose may be related to the reduced irritability and nervousness of the rats, because stress is well known to increase L-DOPA-induced dyskinetic movements in Parkinson’s disease and in parkinsonian animal models (Jankovic, 2005). It is also possible that the increased length of nicotine treatment resulted in the more pronounced reduction in L-DOPA-induced AIMs.

Four weeks later, the dose of L-DOPA was increased to 12 mg/kg to evaluate the effect of nicotine treatment with increased L-DOPA dosing. Despite the increase in L-DOPA dose, the nicotine-induced decline in total AIMs (p < 0.01) was maintained (AIMs III; Fig. 1), due to a significant reduction in oral and forelimb movements (AIMs III; Fig. 2).

**Crossover Study Shows That Removal of Nicotine from the Drinking Water Worsens L-DOPA-Induced AIMs, Whereas Administration of Nicotine to L-DOPA-Primed Rats Reduces AIMs.** The results in Figs. 1 and 2 demonstrate that nicotine administered to rats in the drinking water improves L-DOPA-induced AIMs. As an alternate approach to determine the relationship of these changes in behavior to the nicotine treatment, the rats originally receiving nicotine were switched to saccharin-only drinking water. In contrast, animals initially receiving only saccharin were now provided with nicotine in the drinking water. L-DOPA dosing was continued throughout.

The results in Fig. 3 (left panels) show that nicotine treatment to rats with existing L-DOPA-induced AIMs resulted in a significant reduction in total, oral, and forelimb AIMs. By contrast, removal of nicotine led to an increase in L-DOPA-induced AIMs to levels closer to those observed in non-nicotine-treated rats (Fig. 3, right panels). For these studies, AIM ratings of the same rat were compared before and after nicotine crossover.

**TABLE 1**

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Treatment</th>
<th>Cotinine</th>
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<tbody>
<tr>
<td>Drinking water</td>
<td>Saccharin</td>
<td>0 ± 0</td>
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<tr>
<td></td>
<td>Nicotine</td>
<td>50 µg/ml</td>
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<tr>
<td></td>
<td>Nicotine</td>
<td>25 µg/ml</td>
</tr>
<tr>
<td>Minipump</td>
<td>Vehicle</td>
<td>0 ± 0</td>
</tr>
<tr>
<td></td>
<td>Nicotine</td>
<td>2 mg/kg/day</td>
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Plasma cotinine levels in rats receiving chronic nicotine

Rats were administered nicotine in the drinking water or via minipump. Cotinine levels were measured in rat plasma 2 wks after the initiation of maintenance levels of nicotine in the drinking water or after minipump implantation. Values represent the mean ± S.E.M. of the indicated number of animals.
Nicotine Reduces L-DOPA-Induced AIMs in Rats

Nicotine administration via minipump, yielded a similar improvement in L-DOPA-induced AIMs in rats with unilateral nigrostriatal damage. Four weeks after 6-OHDA lesioning, rats were implanted with minipumps containing vehicle or nicotine (4 Fig. 4). A nicotine dose of 2 mg/kg/day yielded plasma cotinine values of $336 \pm 49$ ng/ml, which were similar to those in the plasma of moderate smokers (Table 1). Minipumps were replaced as necessary to ensure continuous, constant nicotine delivery. Plasma cotinine levels were measured every 2 weeks after the start of minipump implantation and were similar throughout the course of treatment. The rats were subsequently given L-DOPA (8 mg/kg) once daily for 4 weeks and then 12 mg/kg L-DOPA for an additional 3 weeks.

L-DOPA administration to vehicle-treated rats led to the development of AIMs that were maximal by 20 to 80 min after injection and then gradually diminished (AIMs I and II; Fig. 4). Nicotine treatment significantly reduced total AIMs with either the 8 or 12 mg/kg dose of L-DOPA (AIMs I and II; Figs. 4 and 5). There were also significant declines in various AIM subtypes (AIMs I and II; Fig. 5).

Crossover Minipump Study Shows That Removal of Nicotine Worsens L-DOPA-Induced AIMs, Whereas Its Administration to Primed Rats Reduces Their Occurrence. The L-DOPA-treated rats that were originally implanted with minipumps releasing vehicle now received minipumps prefilled with nicotine (2 mg/kg/day). The once daily L-DOPA dosing was continued throughout. After insertion of the nicotine minipumps, there was ~50% reduction in total AIMs (Fig. 6, left panels) in rats with pre-existing AIMs, that is, in L-DOPA-primed animals. The reduction in AIMs seemed to be caused by a significant decrease in abnormal axial and oral movements (Fig. 6, left panels). This nicotine-induced improvement persisted up to 6 weeks, after which time the study was discontinued.

The rats that had initially been implanted with minipumps releasing nicotine were now implanted with new pumps that released only vehicle. Removal of nicotine resulted in a significant increase in total oral, and forelimb AIMs 1 week later (Fig. 6, right panels). These combined data provide further support for the contention that the alterations in AIMs are due to nicotine administration.

Nicotine Treatment Does Not Affect Motor Function in Unilateral 6-OHDA-Lesioned Rats. Several approaches were used to evaluate the effect of nicotine treatment on motor function in rats with a unilateral nigrostriatal lesion. One of these involved measurement of dopaminergic drug-induced rotational behavior using a computerized system (ROTOMAX, AccuScan System; AccuScan Instruments, Inc.) (Mabandla et al., 2004; Howells et al., 2005; Steiner et al., 2006; Lindgren et al., 2007). This test has been extensively used for 6-OHDA-lesioned rats because it is objective, reliable, and provides a good index of dopaminergic denervation (Meredith and Kang, 2006). No difference was observed in amphetamine-induced turning in rats that received vehicle (158 ± 30, n = 9) compared with nicotine (188 ± 27, n = 9). There was also no difference in L-DOPA-induced-turning behavior in rats that received vehicle (11.7 ± 3.1, n = 10) versus those that received nicotine (11.5 ± 4.1, n = 9).

The effect of nicotine treatment was also evaluated on exploratory activity after L-DOPA treatment in the limb use asymmetry or cylinder test. The results in Table 2 show that the percentage of nonimpaired (ipsilateral) limb use on the wall 1 h after L-DOPA treatment was similar in vehicle-
treated rats (41.9 ± 6.3, n = 8) and nicotine-treated (43.5 ± 6.7, n = 8) rats. In addition, impaired (contralateral) limb use was similar in vehicle-treated rats (14.4 ± 4.8, n = 8) and nicotine-treated (17.9 ± 6.1, n = 8) rats. There was also no significant difference in landing after a full rear on the non-impaired side for vehicle (40.5 ± 7.1, n = 8) or nicotine-treated (39.1 ± 9.5, n = 6) rats or in landing on the impaired side for vehicle (32.2 ± 5.4, n = 8) or nicotine-treated (24.9 ± 6.7, n = 6) rats. Simultaneous contact of both the left and right forelimb against the wall was also not influenced by nicotine (vehicle, 44.5 ± 7.9, n = 8; nicotine-treated, 38.6 ± 6.3, n = 6) nor was simultaneous landing after a full rear (vehicle, 28.1 ± 5.6, n = 8; nicotine-treated, 35.3 ± 8.6, n = 6). These combined data suggest that nicotine treatment does not affect behaviors associated with nigrostriatal damage, consistent with our previous studies in monkeys (Quik et al., 2007b).

**Nicotine Treatment Does Not Modify the Striatal Dopamine Transporter.** To determine whether nicotine treatment might influence expression of L-DOPA-induced AIMs, we tested the transporter in the striatum of lesioned L-DOPA-injected rats treated with and without nicotine. In rats that did not receive nicotine, there was a decrease in the striatal dopamine transporter with 6-OHDA lesioning compared with the unlesioned side as expected. Striatal dopamine transporter values in non-nicotine-treated rats were reduced from 7.47 ± 0.11 to 5.12 ± 0.43 fmol/mg (n = 10) with lesioning, with similar values in nicotine-treated lesioned rats (7.69 ± 0.13 and 5.19 ± 0.38 fmol/mg, n = 9, on the unlesioned and lesioned side, respectively). Thus, nicotine exposure does not influence expression of the dopamine transporter either in control or lesioned striatum. The relatively high transporter levels on the lesioned side in the current study probably reflects dopaminergic recovery that develops 6 months after lesioning, as shown previously (Stanic et al., 2003; Lee et al., 2008). Our previous data showed that the current lesioning paradigm results in a ≤80% loss of the striatal dopamine transporter as assessed when the rats are killed ~2 weeks after the lesion.

**Discussion**

The aim of the present study was to determine the effect of nicotine treatment on the development of L-DOPA-induced AIMs in rats with nigrostriatal damage. The results are the first to show that chronic nicotine dosing significantly reduced abnormal movements in this animal model of L-DOPA-induced AIMs. Not only did nicotine pretreatment lead to a decrease in L-DOPA-induced AIMs, but nicotine also reduced established AIMs. Thus, these results extend our previous results in parkinsonian nonhuman primates (Quik et al., 2007b) and provide support for the idea that nicotine may minimize the dyskinesias that arise with L-DOPA treatment in patients with Parkinson's disease.

Another novel aspect of this study shows that nicotine reduces L-DOPA-induced AIMs whether it is administered in a pulsatile/intermittent manner (drinking water) or via constant exposure (minipump). These two modes of nicotine administration differ from each other in that pulsatile exposure (minipump) reduces L-DOPA-induced AIMs to a similar extent as seen with chronic intermittent nicotine administration. However, this is most likely followed by chronic receptor desensitization or inactivation until the nicotine dissipates (Giniatullin et al., 2005; Wang and Sun, 2005). The results showing that constant and intermittent nicotine administration yields a similar antikinetic effect suggest that the decline in L-DOPA-induced AIMs does not depend on the duration and/or extent of desensitization. Alternatively or as well, they may suggest that the degree of desensitization achieved with intermittent dosing contributes maxi-
mally to the beneficial effect of nicotine on aberrant motor behavior. An important question that next arises is whether the effect of nicotine is mediated through nicotinic receptor activation and, if yes, which subtypes might be involved. Continued studies with central nervous system-selective nicotinic receptor subtype blockers or nicotinic receptor subunit knockout mice are required to address this issue.

The present results, combined with previous data (Quik et al., 2007b), indicate that nicotine reduces L-DOPA-induced motor complications in two distinct animal models. L-DOPA-treated lesioned monkeys exhibit dyskinetic-like movements that most closely resemble the abnormal motor movements observed in patients with Parkinson’s disease after L-DOPA treatment (Aubert et al., 2005; Samadi et al., 2005; Quik et al., 2007b). The rat model has been extensively validated using a rating scale that attempts to model the human condition. L-DOPA-induced AIMs in rats are reminiscent of L-DOPA-induced motor abnormalities in Parkinson’s disease in that they are involuntary and can be quite disabling. They also occur uniquely in response to L-DOPA treatment and are not present in L-DOPA-naive animals (Cenci et al., 1998, 2002; Lundblad et al., 2002; Cenci and Lindgren, 2007; Dekundy et al., 2007). Thus, the rat model seems predictive of changes in L-DOPA-treated patients with Parkinson’s dis-

**Fig. 5.** Continuous nicotine exposure via minipump reduced individual AIM subtypes after L-DOPA treatment. Rats were given vehicle (Veh) or nicotine (Nic; 2 mg/kg/day) via minipump and subsequently administered L-DOPA. The rats were evaluated for total, axial, oral, and forelimb AIMs by two raters, one blinded to the treatment status of the animals. The total number of AIMs represents the sum of the three AIM subtypes. Each value represents the mean ± S.E.M. of 10 to 12 rats. *, p < 0.05; **, p < 0.01; and ***, p < 0.001 compared with rats receiving no nicotine using a Mann-Whitney U test.

**Fig. 6.** Nicotine administration reduced L-DOPA-induced AIMs, whereas its removal resulted in an increase in AIM development. Crossover study depicting the effect of constant nicotine exposure via minipump on L-DOPA-induced AIMs. The left panels depict results from rats that were initially implanted with minipumps containing only vehicle (Veh) and then given L-DOPA. They were subsequently implanted with minipumps containing nicotine (Nic), as detailed in the treatment schedule depicted in the top panel. The right panels depict results from rats that had initially been implanted with nicotine-containing minipumps and were then treated with L-DOPA. They were subsequently implanted with minipumps containing only vehicle. Each value represents the mean ± S.E.M. of 10 to 12 rats. *, p < 0.01 and ***, p < 0.001 compared with the initial treatment using a Wilcoxon test.
Nicotine treatment does not affect various measures of motor function in unilateral 6-OHDA-lesioned rats. In experiment A, unilateral 6-OHDA-treated rats were given vehicle or nicotine 2 wks after lesioning and then tested 2 to 3 wks later for rotation in response to amphetamine. In experiment B, unilateral 6-OHDA-treated rats were administered nicotine 3 to 4 wks after lesioning. After a 2-wk nicotine pretreatment period, they then received injections with 8 mg/kg L-DOPA plus benzerazide once daily for 3 to 5 wks with the resultant development of AIMs. They were then tested for L-DOPA-induced turning and different measures of exploratory asymmetric forelimb use (cylinder test) 1 h after L-DOPA administration. The results show there are no significant differences in performance of the various motor measures with nicotine treatment compared with vehicle. Each value represents the mean ± S.E.M. of 6 to 8 rats.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Test</th>
<th>Measure Evaluated</th>
<th>Vehicle-Treated</th>
<th>Nicotine-Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Rotation</td>
<td>Amphetamine-induced turning</td>
<td>158 ± 30</td>
<td>188 ± 27</td>
</tr>
<tr>
<td></td>
<td>Rotation</td>
<td>L-DOPA-induced turning</td>
<td>117.7 ± 3.1</td>
<td>115.5 ± 4.1</td>
</tr>
<tr>
<td></td>
<td>Forelimb use asymmetry or cylinder test</td>
<td>Percentage use of nonimpaired forelimb on wall</td>
<td>41.9 ± 6.3</td>
<td>43.5 ± 6.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Percentage use of impaired forelimb on wall</td>
<td>14.4 ± 4.8</td>
<td>17.9 ± 6.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Percentage simultaneous use of forelimbs on wall</td>
<td>44.5 ± 7.9</td>
<td>38.6 ± 6.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Percentage use of nonimpaired forelimb on landing</td>
<td>40.5 ± 7.1</td>
<td>39.1 ± 9.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Percentage use of impaired forelimb on landing</td>
<td>32.2 ± 5.4</td>
<td>24.9 ± 6.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Percentage simultaneous use of forelimbs on landing</td>
<td>28.1 ± 5.6</td>
<td>35.3 ± 8.6</td>
</tr>
</tbody>
</table>

The mechanisms whereby nicotine reduces 1-DOPA-induced AIMs are currently unknown. Converging evidence suggests that dyskinesias probably arise because of aberrant function at both pre- and postsynaptic sites in the striatum (Aubert et al., 2005; Chen et al., 2005; Carta et al., 2006; Cenci and Lundblad, 2006; Meissner et al., 2006; Cenci, 2007; Cenci and Lindgren, 2007). Studies in 6-OHDA-lesioned rats show that the incidence of AIMs correlates with elevated striatal levels of 1-DOPA, dopamine, and its metabolites, that is, 1-DOPA-treated lesioned animals with higher striatal levels of these compounds develop AIMs, whereas those with lower levels do not (Carta et al., 2006; Cenci and Lundblad, 2006; Meissner et al., 2006; Cenci, 2007). In addition, microdialysis studies show that there is an excessive striatal dopamine release in 1-DOPA-treated lesioned rats with the development of AIMs (Carta et al., 2006). Elevated plasma 1-DOPA levels are also associated with dyskinetic-like movements in parkinsonian monkeys (Pearce et al., 2001). Moreover, a PET study in 1-DOPA-treated patients with Parkinson’s disease showed that the presence of dyskinesias correlated with an increase in dopamine release in striatum (Pavese et al., 2006). Because nicotine treatment has been shown to modulate dopamine release and turnover (Quik et al., 2006), one possibility is that nicotine-induced decline in 1-DOPA-induced AIMs is linked to a decrease in enhanced dopamine release, mediated through nicotinic receptor desensitization.

Postsynaptic transduction mechanisms have also been associated with the development of 1-DOPA-induced abnormal involuntary movements (Cenci and Lundblad, 2006; Cenci, 2007; Cenci and Lindgren, 2007). There seems to be an imbalance in neuronal activity of the direct and indirect output pathways from the striatum, through activation of D1 and inhibition of D2 receptors, respectively. Despite a clear requirement for dopamine receptor stimulation in the development of dyskinesias, there are no consistent changes in the D1, D2, or D3 receptors themselves, suggesting that downstream signaling events linked to dopaminergic receptor stimulation are important. D1 receptors seem more prominently involved than D2 receptors (Picconi et al., 2003; Corvol et al., 2004; Aubert et al., 2005; Guigoni et al., 2005a; Fiorentini et al., 2006), possibly via alterations in G protein coupling, cyclin-dependent kinase 5 (Cdk5), and dopamine cAMP-regulated phosphoprotein (DARPP-32) (Picconi et al., 2003; Aubert et al., 2005). Our previous studies showed that chronic nicotine dosing normalizes aberrant synaptic plasticity in corticostriatal slices after nigrostriatal damage (Quik et al., 2006). Similar mechanisms may be involved in the nicotine-mediated reduction in 1-DOPA-induced dyskinesias.

Nicotine generally exerts its behavioral effects in vivo by interacting with neuronal nicotinic receptors (Gotti et al., 2006; Quik et al., 2007a). These pentameric ligand-gated ion channels may consist only of α-subunits, or of α- and β-subunits. The primary nicotinic receptor subtypes in rat striatum are composed of α4β2 and α6β2 subunits, with the asterisk indicating the possible presence of other subunits in the receptor complex (Gotti et al., 2006; Quik et al., 2007a). The α4β2 receptors are present in the nigrostriatal system as well as other central nervous system regions, whereas α6β2 nicotinic receptors are localized to the dopaminergic nigrostriatal pathway and only a few other brain regions. Taken together, these data suggest that these two subtypes may be particularly relevant to the generation of 1-DOPA-induced AIMs and, as a consequence, the antidyskinetic potential of nicotine. Moreover, because these two subtypes are present only in the brain, they may represent important targets for the eventual development of selective therapies for 1-DOPA-induced dyskinesias.

With respect to future clinical application, our previous studies had shown that long-term intermittent dosing decreased 1-DOPA-induced dyskinesias in parkinsonian monkeys (Quik et al., 2007b). The present results demonstrate that intermittent nicotine dosing is as effective in reducing AIMs in rats as it had been in monkeys. In addition, they show that constant nicotine dosing (minipump) reduces 1-DOPA-induced AIMs in rats. The present data further suggest that nicotine may have potential in the treatment of 1-DOPA-induced dyskinesias in Parkinson’s disease.

References


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