Evaluation of Levodopa Dose and Magnitude of Dopamine Depletion as Risk Factors for Levodopa-Induced Dyskinesia in a Rat Model of Parkinson’s Disease

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ABSTRACT

Levodopa dose and severity of Parkinson’s disease (PD) are recognized risk factors for levodopa-induced dyskinesia (LID) in humans. The purpose of the present study was to evaluate the ability of these variables to predict severity of LID in a rat model of PD. Varied concentrations of 6-hydroxydopamine were injected into the midbrain to produce wide ranges of dopamine depletion in striatum. Three weeks later, rats were given daily injections of levodopa (2–10 mg/kg i.p.) plus benserazide (12.5 mg/kg i.p.) for 15 days. Abnormal involuntary movements (AIMs) were measured for limb, axial, orolingual, and rotatory movements. Dose-response analysis for total AIM scores yielded a levodopa ED50 value of 3.2 mg/kg on treatment day 15. There were strong interrelated correlations between individual AIM categories (ρ > 0.7) and for each AIM category in regard to total AIM score (ρ > 0.7). In rats that received levodopa doses that were greater than the ED50, rates of amphetamine-induced rotation were significantly correlated with total AIM scores (ρ = 0.413). However, of those rotating >5 times/min, 34% had relatively low AIM scores (<8). Likewise, there was a significant correlation between percentages of tyrosine hydroxylase (TH) loss and total AIM scores (ρ = 0.388). However, in those rats that had >85% TH loss, 30% had AIM scores <8. Our results show that given an adequate dose and magnitude of striatal dopamine depletion, levodopa produces dyskinesia with a continuous spectrum of severity. Although levodopa dose and level of dopamine depletion are significant risk factors for LID, we conclude that other factors must contribute to LID susceptibility.

The tremor, rigidity, and akinesia of Parkinson’s disease (PD) are caused by progressive loss of dopamine innervation in the basal ganglia. Symptoms of PD can be largely alleviated by treatment with the dopamine precursor levodopa. However, chronic treatment is often complicated by the emergence of levodopa-induced dyskinesia (LID), which is characterized by involuntary choreiform or dystonic movements of the face, trunk, or limbs. After 1 year of levodopa treatment, more than 60% of PD patients reportedly show signs of LID, and new cases occur at an incidence of 10% per year (Grandas et al., 1999). Risk factors for LID include young age of PD onset, duration and dose of levodopa treatment, severity and duration of PD symptoms, and female gender (Nutt, 1992; Schrag and Quinn, 2000; Zappia et al., 2005). However, it is also clear that not all PD patients develop LID despite the presence of multiple risk factors. Because LID can severely limit the usefulness of levodopa treatment (Hurtig, 1997), this has prompted much research aimed at discovering mechanisms that underlie the development of LID in PD.

In the last few decades, there has been steady advancement in using the hemi-parkinsonian rat to investigate LID. In this model of PD, rats receive a unilateral intracerebral injection of 6-hydroxydopamine (6-OHDA) that causes ipsilateral destruction of dopamine-containing neurons (Ungerstedt and Arbuthnott, 1970). Because of chronic hemidepletion of dopamine, rats will rotate contralateral to the lesion.

ABBREVIATIONS: PD, Parkinson’s disease; LID, levodopa-induced dyskinesia; 6-OHDA, 6-hydroxydopamine; AIM, abnormal involuntary movement; SNC, substantia nigra zona compacta; ANOVA, analysis of variance; TH, tyrosine hydroxylase; OD, optical density; MFB, median forebrain bundle; PBS, phosphate-buffered saline; LOWESS, locally weighted least-squares smoothing function.
following injections of dopamine agonists such as apomorphine and levodopa, and they rotate in the ipsilateral direction in response to the indirect dopamine agonist amphetamine (Ungerstedt, 1971). Because dopamine agonists are useful clinically in the treatment of PD, the induction of contralateral circling might be interpreted as a therapeutic, anti-parkinsonian effect, but clearly, prolonged rotatory behavior is not as normal or purposeful as one might expect from a “therapeutic” action. Moreover, repeated administration of a dopamine agonist is well known to cause behavioral sensitization that augments circling behavior (Morelli et al., 1989). These considerations have prompted the suggestion that agonist-induced circling behavior may more accurately model dyskinesia than a therapeutic anti-parkinsonian effect (Henry et al., 1998; Konitsiotis and Tsironis, 2006). Nevertheless, it is also clear that repetitive circling in a rat is a poor model of the complex, dyskinetic movements that characterize LID in humans. Thus, the interpretation of circling behavior in the hemi-parkinsonian rat is controversial.

More recently, a rodent behavioral rating scale developed by Cenci et al. (1998) has gained widespread acceptance as a model of LID. Using unilateral 6-OHDA-lesioned rats, levodopa-induced abnormal involuntary movements (AIMs) are rated on a scale of 0 to 4 for each of four categories: 1) limb dyskinesia, 2) axial dystonia, 3) orolingual movements, and 4) rotational behavior. However, a review of published studies using the AIM rating scale produces a confusing picture of the incidence of LID in hemi-parkinsonian rats, with some studies reporting that virtually all rats show significant dyskinesia after chronic levodopa treatment (Bishop et al., 2006; Konitsiotis and Tsironis, 2006; Lindgren et al., 2007), whereas others report that approximately half either do or do not develop LID in an all-or-nothing fashion (Cenci et al., 1998; Picconi et al., 2003; Taylor et al., 2005; Carta et al., 2006). The reason for these different outcomes is not clear, but the dose of levodopa and magnitude of dopamine depletion are important variables in LID that have not been adequately characterized thus far. Therefore, the present studies were undertaken to investigate the influence of these variables in the incidence and severity of LID in unilateral 6-OHDA-lesioned rats.

Materials and Methods

Animals and Surgery. Male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 275 to 325 g at the time of surgery were housed two to a cage in a room with a 12-h light/dark cycle. Rats had ascorbic acid at concentrations that ranged from 1.0 to 11.4 mg/kg i.p.) was given 30 min before 6-OHDA infusions. During recovery from anesthesia, rats were given buprenorphine (0.05 mg/kg s.c.) for analgesia and placed under a warming lamp.

Behavioral Measurements. Three weeks after surgery, rats were placed in clear polycarbonate boxes (28 cm², height 19 cm) and given a single injection of d-amphetamine sulfate (5 mg/kg i.p.). Beginning 30 min after injection, ipsilateral rotations were counted every minute for at least 15 min until no apparent increase in the number of rotations per minute was observed. This procedure ensured that we captured peak rotation rate for each rat. Rotation rate (turns per minute) was calculated as the average of the highest scores recorded over a 10-min period. Starting the day after amphetamine treatment, rats received levodopa methyl ester (2, 3, 4, 6, or 10 mg/kg i.p.) or saline (1 ml/kg i.p.) every day for 15 days. These doses of levodopa were chosen because others have shown that similar doses were the minimum necessary to improve akinesia in the hemi-parkinsonian rat (Chang et al., 1999; Lundblad et al., 2002). We adapted the AIM behavioral rating scale of Cenci et al. to assess LID (Lee et al., 2000; Winkler et al., 2002). AIMs were rated on a 0- to 4-point scale for limb, axial, orolingual dyskinesia, and rotational behavior following levodopa or saline treatment. Limb dyskinesia appeared as repetitive myoclonic movements or dystonic posturing of the forelimb contralateral to the lesion. Axial movements were recognized as a lateral flexion or axial rotation of the neck or trunk toward the contralateral side. Orolingual dyskinesia consisted of repetitive chewing movements of the jaw with or without tongue protrusion. Rotational behavior was defined as turning or rotation movement in the direction contralateral to the 6-OHDA lesion. A score of “0” indicated the absence of abnormal movement, “1” indicated an infrequent AIM that occurred <50% of the time, “2” referred to an occasional or frequent AIM occurring >50% of the time, “3” indicated an event that occurred constantly but could be interrupted by tapping the cage with a pen, and “4” referred to a prominent, constant, and uninterruptible AIM. AIMs for each rat were evaluated during a 1-min observation period. AIMs were expressed either as a score for each individual AIM category, with a maximum value of 4, or as a total AIM score calculated as the sum of all four individual AIM scores.

Tyrosine Hydroxylase Immunohistochemistry. The day after the last levodopa or saline treatment, rats were euthanized under isoflurane anesthesia in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The brain was rapidly removed and placed in ice-cold 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS; pH 7.4) for 24 h and then transferred to 30% sucrose in 0.1 M PBS for at least 3 days. Coronal sections through the striatum (40-μm thick) were cut using a freezing microtome and stored in 0.1 M PBS containing 0.1% sodium azide. For immunohistochemistry, free-floating tissue sections were rinsed in PBS (3 × 10 min) while gently agitated. Sections were then placed in 1.5% H₂O₂ for 30 min to reduce endogenous peroxidase activity. Afterward, the tissue was rinsed in PBS (3 times for 10 min) and then blocked in PBS containing 4% horse serum and 0.1% Triton X-100 for 2 h. A mouse anti-tyrosine hydroxylase antibody (1:10,000; Sigma-Aldrich Corp., St. Louis, MO) was added to the block and incubated overnight at 4°C. Next, sections were rinsed in PBS (3 × 10 min) at room temperature and placed in PBS containing 4% horse serum and biotinylated anti-mouse IgG secondary antibody (1:200; Vector Laboratories, Burlingame, CA). This was followed by a PBS rinse (3 × 10 min) and equilibration for 1 h in avidin-biotin complex (ABC; Vector Laboratories). Sections were transferred to a 50 mM Tris buffer solution and treated with diaminobenzidine (Vector Laboratories). Tyrosine hydroxylase (TH) immunoreactivity was visualized using a Leica DM LFS microscope (Wetzlar, Germany), and the optical density (OD) of a rectangular area (1.4 × 1.0 mm) was quantified using a Leica DFC 480 camera and Image Pro-Plus 4.1 software (Media Cybernetics, Inc., Bethesda, MD) run on a personal computer. The percentage of TH loss was calculated by comparing the OD in lesioned dorsolateral striatum with the OD on the nonlesioned side. Measurements were corrected for TH staining due to
noradrenergic fibers and nonspecific background immunoreactivity by subtracting the OD in adjacent cerebral cortex from that in dorsolateral striatum on each side of the brain (Meshul and Allen, 2000).

**Drugs.** Levodopa methyl ester, 6-OHDA HBr, d-amphetamine sulfate, benserazide, and desipramine HCl were obtained from Sigma-Aldrich Corp. Buprenorphine HCl was obtained from Reckitt and Coleman (Hull, UK), and isoflurane was from Hospira (Lake Forest, IL).

**Statistics.** Levodopa time-effect data were analyzed for statistical significance using two-way analysis of variance (ANOVA) with repeated measures followed by a Holm-Sidak pairwise comparison test. Dose-response data were plotted and ED50 values were calculated using a four-parameter Hill equation. Each dose group was compared with the saline-treated group with a Mann-Whitney rank sum test. Correlations between AIM scores and dopamine depletion measures were performed with a nonparametric Spearman’s rank-order correlation test. A locally weighted least-squares smoothing function (LOWESS) was used to fit a locally weighted regression curve to the data without presupposing any particular functional relationship, such as linear or quadratic. Data analysis was done using either SigmaStat or SYSTAT (Systat Software, Inc., San Jose, CA) run on a personal computer.

**Results**

**Correlation between Amphetamine Rotations and TH Loss.** To produce rats with varying magnitudes of TH depletion, we injected concentrations of 6-OHDA that ranged from 1.0 to 11.4 μg/μl. The photomicrographs in Fig. 1 show examples in which a low concentration of 6-OHDA (1.0 μg/μl) reduced TH staining in striatum by 53.6%, whereas a higher concentration (6 μg/μl) reduced TH staining by 97.8%. By producing rats with varying degrees of TH loss, we were able to study the relationship between magnitude of TH loss in striatum and peak rate of rotations produced by amphetamine (5 mg/kg). As shown in Fig. 2, we found a significant correlation between magnitude of TH loss and peak rate of amphetamine-induced rotations (n = 90; Spearman’s ρ correlation = 0.52, P = 0.0000001). Data in Fig. 2 were plotted using the logarithm of rotations per minute to produce a more linear relationship, as can be seen from the LOWESS curve. The vertical dashed line in Fig. 2 indicates 85% loss of TH immunoreactivity, which is the threshold level of dopamine depletion that has been associated with signs of parkinsonism in cases of idiopathic PD (Kish et al., 1988) and in animal models of PD (Chang et al., 1999; Bezard et al., 2001). The horizontal line in Fig. 2 indicates an amphetamine-induced rotation rate of 5 turns/min that has been shown by others to correlate with a level of dopamine depletion sufficient to cause signs of hemi-parkinsonism in rats (Chang et al., 1999). Although Fig. 2 shows that a significant correlation exists between TH loss and rate of rotation, it also shows that some rats that rotated >5 turns/min had TH loss that was <85%. These data suggest that the rate of amphetamine-induced rotations has a limited ability to predict magnitude of TH loss in striatum.

**Time Course of LID.** To establish the time course of LID, we treated rats with either 10 mg/kg levodopa plus 12.5 mg/kg benserazide (n = 6) or saline (n = 3) every day for 15 days. AIMs were measured every 20 min for 3 h after each injection. To minimize the possible influence of variations in magnitude of dopamine depletion on time course, we excluded rats that showed amphetamine-induced rotations that were <5 turns/min and those that were subsequently shown to have <85% TH loss in striatum. Furthermore, as

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**Fig. 1.** Photomicrographs of 40-μm coronal brain sections showing TH immunolabeling of the striatum. A 6-OHDA concentration of 6 μg/μl caused a 97.8% reduction in TH immunoreactivity (A), whereas a 6-OHDA concentration of 1 μg/μl reduced TH by 53.6% (B). Each rat received injections of 6-OHDA (2-μl volumes) into the SNC and MFB. Cerebral cortex adjacent to dorsolateral striatum was used to correct for nonspecific background staining. Calibration bar represents 1 mm.

**Fig. 2.** Correlation between peak rate of amphetamine-induced rotation and magnitude of TH depletion (n = 90). Dashed lines indicate an amphetamine-induced rotation rate of 5 turns/min and an 85% loss of TH staining in striatum. A LOWESS function provides a relatively linear regression plot, and a Spearman’s correlation between TH loss and amphetamine rotations is highly significant (P = 0.000001). Note that although TH loss of >85% was always associated with rates of rotation >5 turns/min, many rats had significant rates of rotation despite TH loss <85%.
we were interested in only those rats that showed significant AIM scores, one levodopa-treated rat with low AIM scores was excluded. As shown in Fig. 3, AIM scores reached their peak 60 min after levodopa, and they declined to zero 3 h after injection. Moreover, a pairwise comparison test (Holm-Sidak method) showed that the average AIM score at 20 min was significantly greater at treatment day 15 compared with day 1 (P < 0.05). AIM scores evoked by levodopa on days 1 and 15 were significantly greater than scores from saline-treated rats, which had an average score of 0. These data show that chronic levodopa treatment shortens the onset latency and increases the magnitude of dyskinesia. Increased peak effect and shortened latency are features of levodopa sensitization that have been described previously for therapeutic effects of levodopa in patients with PD (Nutt et al., 1992) and for levodopa-induced circling behavior in hemiparkinsonian rats (Mura et al., 2002). Although others have also described reduced duration of levodopa effect after chronic treatment (Papa et al., 1994), our data do not support this conclusion.

**Levodopa Dose Responses.** Next, we examined dose-dependent effects of levodopa on AIM scores. Rats were given daily injections of either saline or levodopa at doses of 2, 3, 4, 6, or 10 mg/kg plus 12.5 mg/kg benserazide for 15 days. AIM scores were measured at the time of peak levodopa effect (60 min after each injection). To minimize the possible influence of large variations in level of dopamine depletion, we excluded rats that showed amphetamine-induced rotations that were <5 turns/min and those that were subsequently shown to have <85% TH loss in striatum. As shown in Fig. 4A, levodopa at daily doses of 3, 4, and 6 mg/kg resulted in total AIM scores that were significantly increased on day 15 compared with day 1 of treatment (n = 8 per group; P < 0.01, two-way ANOVA with repeated measures, Holm-Sidak method). Although the 10-mg/kg dose also showed a time-dependent increase in AIM scores (n = 11; P = 0.05), the relatively low rate of sensitization might be due to a ceiling effect caused by high AIM scores at the start of chronic treatment. In contrast, rats treated with the 2-mg/kg dose showed no significant increase in AIM scores over the duration of treatment (n = 5; P < 0.05). These data suggest that sensitization to dyskinetic effects of levodopa is most prominent at levodopa doses between 3 and 8 mg/kg. Moreover, scores for all four individual AIM categories gradually increased over time (Supplemental Table 1). In contrast, rats treated daily with saline had consistently low AIM scores with an average of “0.1” (n = 31).

Figure 4B shows the average levodopa dose-effect curve on AIM scores measured 60 min after injection on day 15 of chronic treatment. Using a four-parameter Hill equation, we estimated the levodopa ED50 value at 3.2 mg/kg (n = 40). All but the 2-mg/kg dose produced a significant increase in AIM scores compared with the saline group (P < 0.05, Mann-Whitney rank sum test). Data were fitted to a four-parameter Hill equation, with an estimated ED50 value of 3.2 mg/kg. Data are from rats that rotated ≥5 turns/min in response to amphetamine and had ≥85% loss of TH in striatum.

![Fig. 3. Time course for AIM scores following levodopa. Rats were treated daily with 10 mg/kg levodopa plus 12.5 mg/kg benserazide for 15 days. AIM scores reach maximum values 60 min after levodopa. Asterisks indicate times at which AIM scores on treatment day 15 are significantly higher than scores on treatment day 1 (n = 5; P < 0.05, two-way ANOVA with repeated measures, Holm-Sidak method).](image-url)

![Fig. 4. Levodopa dose responses. A, AIM scores as a function of treatment day for different doses of levodopa. AIM scores were significantly increased at day 15 compared with day 1 for rats that received daily doses of 3, 4, and 6 mg/kg levodopa (n = 8 for each group; P < 0.01, two-way ANOVA with repeated measures, Holm-Sidak method). Error bars are not shown for clarity. B, average levodopa dose-response curve for total AIM scores measured on treatment day 15 (n = 40). Asterisks indicate significant increases in AIM scores compared with the saline group (P < 0.05, Mann-Whitney rank sum test). Data were fitted to a four-parameter Hill equation, with an estimated ED50 value of 3.2 mg/kg. Data are from rats that rotated ≥5 turns/min in response to amphetamine and had ≥85% loss of TH in striatum.](image-url)
improved when rats with striatal TH loss below the median (90.4%) were excluded from the analysis ($n = 27; \rho = 0.57, P = 0.001$). These data clearly show that dose is a significant risk factor for the development of LID.

**Effects of Levodopa on Individual AIM Categories.** Although data presented thus far describe effects of levodopa on total AIM scores, we also considered the possibility that dose-dependent effects of levodopa might differ among individual AIM categories. Therefore, we constructed levodopa dose-response curves for each AIM category, as seen in Fig. 5. Levodopa at a dose of 3 mg/kg or higher caused significant increases in AIM scores for limb (Fig. 5A) and orolingual (Fig. 5C) dyskinesias, whereas a dose of 4 mg/kg or higher was required for significant increases in axial dyskinesia (Fig. 5B) and rotational behavior (Fig. 5D; $P < 0.05$, Mann-Whitney rank sum test). Plotting these data with the Hill equation yielded estimates of $ED_{50}$ values that ranged from 2.7 mg/kg for limb dyskinesia to 3.8 mg/kg for axial dyskinesia. These data suggest that levodopa has similar dose-dependent effects on each of the individual AIM categories. This impression was confirmed by performing a nonparametric correlation analysis between all individual AIM categories. As shown in the scatter plots in Fig. 6A, all four AIM variables are closely correlated with each other, and Fig. 6B shows that scores in each AIM category are highly correlated with total AIM scores as well (Spearman’s $\rho \geq 0.70, P < 0.05$). Although these data suggest that individual AIM categories are highly interrelated, this does not imply that each AIM category represents the same phenomena.

**Amphetamine-Induced Rotations and TH Loss As Predictors of LID.** In an effort to evaluate the predictive values of amphetamine-induced rotation rate and magnitude of TH loss, we plotted these variables as a function of AIM scores measured on day 15 of treatment. In these analyses, we included all rats regardless of amphetamine rotation rate and magnitude of TH loss. However, because our data showed that LID is dose-dependent, we did not want to include data from rats in which the dose of levodopa might be too low to evoke LID. Therefore, we excluded animals treated with doses of levodopa that were at or below the $ED_{50}$ value of 3 mg/kg. Figure 7A shows that the logarithm of amphetamine-induced rotations per minute was significantly correlated with total AIM scores ($n = 41; \text{Spearman’s } \rho = 0.41, P = 0.008$). The fact that only one rat with a rotation rate $>5$ turns/min had significantly elevated AIM scores in the range of 12 to 16 suggests that 5 rotations/min is a reasonable cutoff value to screen for rats that are susceptible to LID. However, one should note that 34% of all rats with an amphetamine rotation rate $>5$ turns/min had relatively little dyskinesia with AIM scores that were $<8$ (12 of 35 rats). Likewise, the plot of TH loss versus total AIM scores in Fig. 7B shows a significant correlation as evaluated by nonparametric correlation analysis ($n = 41; \text{Spearman’s } \rho = 0.39, P = 0.012$). However, 30% of all rats with $>85\%$ TH loss had relatively little dyskinesia with AIM scores that were $<8$ (8 of 27 rats). These data suggest that magnitude of TH loss and rate of amphetamine-induced rotations are only weak predictors of LID presence or absence.
Discussion

Results from our studies clearly show that LID is related to dose and duration of levodopa treatment. We also found that both amphetamine-induced rate of rotation and percentage loss of TH immunoreactivity in striatum were positively correlated with magnitude of LID. In particular, rats that had greater than 85% loss of striatal TH and rotated in response to amphetamine at >5 turns/min increased the likelihood of having high AIM scores. However, many rats had low AIM scores despite adequate levodopa dose and evidence of high levels of dopamine depletion. We conclude that significant dopamine depletion, as indicated by TH loss or rotation rate, is necessary but not sufficient for the manifestation of LID. Therefore, factors other than levodopa dose and level of dopamine depletion must significantly influence LID susceptibility.

It is reasonable to consider the possibility that each AIM category might not be equally useful as a measure of LID. For example, Boulet et al. (2006) reported that the threshold for evoking orolingual dyskinesia was much lower than the thresholds for evoking axial dyskinesia, abnormal limb movements, or rotational behavior during deep brain stimulation of the subthalamic nucleus in rats. In addition, the dopamine agonist bromocriptine reportedly fails to evoke axial, limb, or orolingual dyskinesia despite causing circling behavior in unilaterally dopamine-depleted rats (Lundblad et al., 2002). Furthermore, several studies have reported that a variety of pharmacological agents display differential abilities to modify the expression of these individual AIMS (Lundblad et al., 2002; Taylor et al., 2005, 2006). On the other hand, results from our present studies showed good correlations between scores for levodopa-induced axial, limb, orolingual, and rotational movements. Moreover, the levodopa ED50 values for individual AIM categories were similar, which suggests that there is no significant difference in potency for inducing each abnormal movement. Therefore, our data suggest that the four AIM categories are inherently interrelated. Nevertheless, this does not necessarily mean that each AIM category is measuring the same phenomena. We suggest one should be cautious about combining all AIM scores into a unified score.

As has been reported by others, we found that magnitude of TH loss in striatum was well correlated with rate of amphetamine-induced rotations (Hefti et al., 1980; Papa et al., 1994; Chang et al., 1999). This was expected, as both parameters are markers of the magnitude of unilateral dopamine depletion. However, note that many subjects with rotations >5 turns/min had relatively low AIM scores. Nevertheless, a significant number of subjects with TH loss ≥85% had relatively low AIM scores.

Fig. 6. Correlational scatter plots for individual AIM categories measured on day 15 of treatment with 2, 3, 4, 6, or 10 mg/kg levodopa. A, each AIM category shows a significant correlation with every other category (n = 40; Spearman’s correlation ρ ≥ 0.70, P < 0.05). B, each AIM category shows a significant positive correlation with total AIM scores (P < 0.05). Regression curves in all figures are calculated with a LOWESS function; Spearman’s correlation was used to test for statistical significance. The plotted data points have been jittered to show all data points with the same coordinates.

Fig. 7. Relationships between peak amphetamine-induced rotation rate and magnitude of TH depletion on AIM scores measured on day 15 of levodopa treatment. Data are shown only for rats that received doses of levodopa that were above the ED50 (4, 6, or 10 mg/kg). A, graph shows a significant correlation between rate of amphetamine-induced rotations and AIM scores (n = 41; Spearman’s correlation ρ = 0.41, P = 0.008). However, note that many subjects with rotations >5 turns/min had relatively low AIM scores. B, graph shows a significant correlation between loss of TH immunostaining in striatum and AIM scores (n = 41; Spearman’s correlation ρ = 0.39, P = 0.012). Nevertheless, a significant number of subjects with TH loss ≥85% had relatively low AIM scores.
depletion. On the other hand, the fact that TH loss and amphetamine-induced rotations were only weakly correlated with AIM scores suggests that magnitude of dopamine depletion is only a modest predictor of LID susceptibility. It is pertinent to emphasize that few rats with amphetamine rotation rates <5 turns/min or with TH loss <70% had significantly elevated AIM scores; these data suggest that a level of dopamine depletion that produces parkinsonism is necessary but not sufficient to confer susceptibility to LID. However, the fact that many rats that had marked TH loss or high levels of amphetamine-induced rotation failed to show significant LID, despite adequate levodopa dose, suggests that other variables contribute to LID susceptibility. There is some evidence to suggest that alterations in dopamine receptor-coupled G proteins may confer susceptibility to LID (Corvol et al., 2004; Aubert et al., 2005; Kovoor et al., 2005). Other studies have linked LID to alterations in N-methyl-D-aspartate receptor subunit expression or phosphorylation state (Oh et al., 1998; Hallett et al., 2005; Gardoni et al., 2006). Finally, LID susceptibility has also been associated with relatively high levels of levodopa in striatum (de la Fuente-Fernández et al., 2004; Carta et al., 2006) that may be caused by alterations in the blood-brain barrier (Westin et al., 2006). Further work will be needed to identify the factors other than levodopa dose and magnitude of dopamine depletion that contribute to LID susceptibility.

A review of the literature produces a confusing picture of the incidence of LID in the rat model of PD. Given an adequate dose and magnitude of striatal dopamine depletion, our results show that levodopa produces dyskinesia with a continuous spectrum of severity in hemi-parkinsonian rats. This finding agrees with the results of several other studies (Andersson et al., 1999; Lee et al., 2000; Soghomonian, 2006). But other studies have reported that virtually all rats with sufficient levels of dopamine depletion develop signs of LID as measured by elevated AIM scores. However, the high incidence of LID in some studies might be attributed to the use of supratherapeutic doses of levodopa that are given for longer than the 15-day period used in our present study (Bishop et al., 2006; Lindgren et al., 2007). Furthermore, others have reported that severe dopamine depletion as measured by >95% loss of TH-positive cells in the SNC allows LID to appear in all rats (Winkler et al., 2002; Paillé et al., 2004). In contrast, several other studies have reported that chronic treatment with “therapeutic” doses of levodopa (6–10 mg/kg) evokes approximately equal numbers of dyskinetic and nondyskinetic rats in an all-or-nothing fashion (Cenci et al., 1998; Picconi et al., 2003; Taylor et al., 2005; Carta et al., 2006). Because these all-or-nothing reports of LID incidence in hemi-parkinsonian rats, it is still possible that genetic differences may exert an influence. In addition, magnitude of dopamine depletion in nonstriatal basal ganglia, such as the subthalamic nucleus, may be an important variable in LID susceptibility (Soghomonian, 2006).

In summary, our data show that both levodopa dose and level of striatal dopamine depletion are significant variables in the rodent model of LID. However, neither rate of amphetamine-induced rotation nor magnitude of striatal TH loss can accurately predict AIM scores despite adequate levodopa dose. Furthermore, given adequate dose and level of dopamine depletion, we found that chronic levodopa treatment results in a continuous spectrum of LID severity in hemi-parkinsonian rats. Future studies will be necessary to better define variables that contribute to LID susceptibility.

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