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L-Dihydroxyphenylalanine modulates the steady-state expression of mouse striatal tyrosine hydroxylase, aromatic L-amino acid decarboxylase, dopamine and its metabolites in an MPTP mouse model of Parkinson's disease

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Abstract

Aims—L-3,4-Dihydroxyphenylalanine (L-DOPA) is the most effective symptomatic treatment for Parkinson's disease (PD), but PD patients usually experience a successful response to L-DOPA therapy followed by a progressive loss of response. L-DOPA efficacy relies on its decarboxylation by aromatic L-amino acid decarboxylase (AAAD) to form dopamine (DA). So exogenous L-DOPA drives the reaction and AAAD becomes the rate limiting enzyme in the supply of DA. In turn, exogenous L-DOPA regulates the expression and activity of AAAD as well as the synthesis of DA and its metabolites, changes that may be linked to the efficacy and side-effects of L-DOPA.

Main Methods—One-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse PD model was utilized to study the effects of L-DOPA on the steady-state level and activity of AAAD, tyrosine hydroxylase (TH), DA and the metabolites of DA. The MPTP and control mice were treated twice daily with PBS or with 100 mg/kg of L-DOPA for 14 days and the expression and activity of AAAD, the expression of TH and the levels of DA and its metabolites were determined 24 hrs after L-DOPA or PBS treatment, when exogenous L-DOPA is eliminated.

Key Findings—In the MPTP model, L-DOPA reduced the steady-state expression and the activity of striatal AAAD by 52% and 50%, respectively, DA and metabolites were also significantly decreased.

Significance—The outcome shows that while L-DOPA replenishes striatal DA it also down-regulates AAAD and the steady-state synthesis and metabolic capability of the dopaminergic system. These findings are important in the precipitation of L-DOPA induced side effects and the management of L-DOPA therapy.

Keywords

Parkinson's disease; L-DOPA therapy; L-DOPA side effects; Dyskinesia; L-3,4-dihydroxyphenylalanine; aromatic L-amino acid decarboxylase

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INTRODUCTION

L-3, 4-dihydroxyphenylalanine (L-DOPA), the precursor of dopamine (DA), is the most effective drug for treating the symptoms of Parkinson's disease (PD). The therapeutic benefit of L-DOPA is believed to result from its conversion by aromatic L-amino acid decarboxylase (E.C. 4.1.1.28, AAAD) to DA (Calne and Karoum 1969; Goodman et al. 2006). When administered, L-DOPA corrects the striatal DA depletion (Jankovic and Tolosa 2007). Although it remains the most effective anti-Parkinson agent available, after years of L-DOPA treatment there is a gradual wearing off of each dose (Cenci and Lindgren 2007), so that late failure of oral L-DOPA treatment occurs of yet unknown mechanism (Kuschinsky and Hornykiewicz 1974; Mouradian et al. 1987; Colosimo and De Michele 1999).

Since L-DOPA is an endogenous intermediate catecholamine molecule its administration can cause drastic changes in the catecholamine system. So, the utility of L-DOPA in supplying DA to the DA-deficient brain of the PD patient can correct most of the PD symptoms, but it can also create an imbalance in the metabolic pathway of the catecholamine. In previous studies it was shown that the sub-chronic administration of L-DOPA increased the activities (Benson et al. 1993; Zhao et al. 2001) as well as the expression (Zhao et al. 2001) of catechol-O-methyltransferase (COMT) and methionine adenosyl transferase (MAT). It was also shown that large doses of L-DOPA resulted in a shorter latency, higher incidence and increased severity of dyskinesia (Lancaster et al. 1973), which helped to link dyskinesia to the dosage of L-DOPA. In addition, L-DOPA therapy results in fluctuations of the serum levels of DA (Chase 1998; Stocchi 2009), that was proposed to be related to the intermittent treatment doses of L-DOPA and which may help to explain the motor complications that occur following L-DOPA therapy (Mouradian et al. 1987; Chase 1998). Many studies have made progress in understanding the limitations to chronic administration of L-DOPA for treatment of motor deficits in Parkinson's disease which relate to drug absorption, metabolism, access to the brain, and response. Changes in dopamine need and in L-DOPA disposition both accompany progression of the disease and L-DOPA treatment. First, L-DOPA availability from oral administration requires its absorption in the small intestine, but PD itself slows gastric motility (Edwards et al. 1992) and with L-DOPA treatment, stimulation of dopamine receptors in the stomach may further depress gastric motility. Blocking peripheral metabolism of L-DOPA that is absorbed is always part of L-DOPA therapy, achieved by the co-administration of carbidopa.

Competition between large neutral amino acids that can be found in a high protein diet and L-DOPA for uptake at the blood brain barrier can also confound efficacy in response to L-DOPA therapy (Leenders KL. 1986). Changes in response to dopamine with chronic and large doses of exogenous L-DOPA also can occur. In normal individuals with dopaminergic pathways intact, DA receptors are continuously exposed to released DA. With PD, there is discontinuous activation of DA receptors, and fluctuations in DA that accompany L-DOPA dosing regimens further destabilize the basal ganglia network responsible for controlling voluntary movement (Chase et al. 1998; Stocchi F 2008).

In the present study, we investigate whether sub-chronic administration of L-DOPA results in modulating the activity and expression of the enzyme AAAD, the expression of tyrosine hydroxylase and the levels of DA and its metabolites. The results show that L-DOPA treatment down-regulates the expression and activity of AAAD, the expression of TH and the striatum exhibits a reduced capacity to produce DA, DOPAC and HVA.

EXPERIMENTAL PROCEDURES

Animals and Treatments

Experiments were performed using male C57Bl/6J mice, weighing 20-25 gm purchased from Jackson Laboratories (Bar Harbor, MA). The animal usage was approved by the Institutional Laboratory Animal Care and Use Committee of Meharry Medical College. The mice were housed 4 per cage under a 12 hr light and 12 hr dark cycle from 6AM to 6PM and 6PM to 6AM in a temperature-controlled room with standard food and water ad libitum. After acclimatization, the mice were treated with the PBS 1 ml/100 gm or with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) at 30 mg/kg i.p. daily for 7 days. MPTP induces selective lesioning of dopaminergic nigrostriatal neurons and produces the PD model. This regimen of MPTP results in a severe depletion of striatal DA and loss of dopaminergic cell bodies in the substantia nigra. Seven days post-MPTP was allowed for the development of the lesion. At the end of the seven day period the MPTP and the PBS control mice were treated with PBS or with L-DOPA, 100 mg/kg (Sigma-Aldrich, St. Louis, MO) i.p., twice daily for 14 days. One group of mice were sacrificed 24 hrs after the last dosage of L-DOPA or PBS, to eliminate the influence of L-DOPA and another group of animals were sacrificed immediately after the last dosage of L-DOPA to assess its acute effects. The animals were sacrificed by decapitation, and cortex, midbrain, hippocampus, striatum and cerebellum were dissected for the analyses of AAAD, TH, DA and its metabolites, DOPAC and HVA. All brain tissue sections (i.e. cerebellum, cortex, hippocampus, striatum and midbrain) were analyzed but no significant differences were found for AAAD, TH, DA and its metabolites, DOPAC and HVA, in the cerebellum, cortex, hippocampus and midbrain for MPTP-treated versus control animals. Thus, only data for striatal AAAD, TH, DA and its metabolites, DOPAC and HVA are presented.

Procedures for AAAD Activity

AAAD activity was measured by the method of Hadjiconstantinou et al. (1993). The procedure involved measuring the product DA derived from the decarboxylation of L-DOPA by the AAAD containing tissue homogenate. Tissue was weighed and homogenized in ice-cold 0.25 M sucrose in a 5 time w/v relationship. The protein concentration was assessed using Lowry method. The reaction began by incubating 10 µg of protein with 100 µl of assay buffer pH 7.4 consisting of; 50 mM sodium phosphate buffer, pH 7.2; 0.1 mM EDTA; 0.17 mM ascorbic acid; 1mM β-mercaptoethanol; 100 µM pargyline; 170 µM ascorbate; 10 µM pyridoxal 5'-phosphate and 500 µM L-DOPA. The mixture was allowed to react at 37°C for 20 min. The reaction was stopped by adding 80 µl of ice cold 0.525 N HClO₄ containing 100 pmol of 3,4-dihydroxybenzylamine, as an internal standard. The constituent reactants were frozen at -80 °C for later analysis. Before analysis the contents were thawed and filter-centrifuged through 25µm pore filter. High performance liquid chromatography (HPLC) was used to measure subsequent DA levels using electrochemical detection (EC). AAAD, which catalyzes the decarboxylation of L-DOPA to DA with pyridoxal phosphate as a cofactor, is found in many tissues. In the human brain, as well as in the rodent brain, AAAD activity has proved to be somewhat elusive because it is very low or undetectable. To increase the sensitivity for detection of DA produced, pargyline was added to the assay to inhibit MAO-catalyzed metabolism of DA to form DOPAC. Though it is possible for DA to be metabolized by catechol-O-methyl transferase (COMT) to HVA, this metabolism occurs at a much lower rate than with MAO. To control for the endogenous synthesis of L-DOPA that is known to be able to occur under some circumstances in TH-expressing cells that might be part of our homogenate, we normalized our experimental DA levels with our control homogenates to which no exogenous L-DOPA was added.

DA and Metabolites Measurement

DA, DOPAC and HVA were assessed by EC HPLC system. Homogenate similar to those used for the AAAD assay was immediately mixed with equal volume of 0.2 M HClO₄ containing 0.05 mM sodium bisulfite and the mixture centrifuged to remove the precipitated protein. The supernatant was then removed and filtered as above and a quantity was injected into the EC HPLC system.

The HPLC System

The HPLC system is housed in the Vanderbilt University Analytical Laboratory and consists of Antec Decade II (oxidation: 0.5) electrochemical detector operated at 33° C. Twenty µl samples of the supernatant are injected using a Water 717+ autosampler onto a Phenomenex Nucleosil (5 µ, 100A) C18 HPLC column (150 × 4.60 mm). Biogenic amines are eluted with a mobile phase consisting of 89.5% 0.1M TCA, 10-2 M sodium acetate, 10-4 M EDTA and 10.5 % methanol (pH 3.8). Solvent is delivered at 0.6 ml/min using a Waters 515 HPLC pump. Using this HPLC solvent the following biogenic amines elute in the following order: noradrenaline, MHPG, Adrenaline, DOPAC, Dopamine, 5-HIAA, HVA, 5-HT, and 3-MT (2). HPLC control and data acquisition are managed by Millennium 32 software.

Western Blots

A portion of the sucrose homogenate described previously in the AAAD assay procedure was also used for immunoblotting analysis. Protein concentration was determined by the method of Lowry et al. (1951) with bovine serum albumin as the standard. Tissue lysates containing 30 µg of protein were separated by SDS-polyacrylamide gel electrophoresis, and the proteins were transferred to a Polyvinylidene Fluoride (PVDF) membrane. Membranes containing protein were incubated overnight at 4°C using a blocking buffer consisting of Tris-buffered saline (10 mM Tris-HCl, 0.15 M NaCl, 0.05% tween-20, pH 8.0) containing 5 % non-fat dry milk with rabbit anti-AAAD antibody (Ab1569, Chemicon, Temecula, CA). The membranes were then exposed to the horseradish peroxidase-conjugated secondary antibody (1:1000) at room temperature for 1 hr. with (Santa Cruz Biotechnology, Santa Cruz, CA). Detection of the antigens with Horseradish Peroxidase (HRP) labeled antigens was achieved by Enhanced Chemiluminescence (GE Health, Amersham). Band density was digitized and measured by image analysis (UN-SCAN-IT, Silk Scientific).

Data analysis

Statistical analysis of the enzyme activity and DA and metabolite levels was performed by one- or two-way analysis of variance followed by a Tukey post-test to compare groups or a Student's *t* test. All analysis was done using Graphpad Prism® (Graphpad Software, Inc, San Diego, CA). Values are the mean ± SEM of the indicated number of mice, and represent data from one to two separate testing periods. Results are considered statistically significant when *p* < 0.05.

RESULTS

Striatal TH and DA levels in the MPTP mouse treated with PBS or with L-DOPA

We first examined the expression of tyrosine hydroxylase (TH) since it is the rate limiting enzyme and a key factor in the synthesis of the catecholamine. Moreover, TH depletion occurs in PD and its analysis becomes a marker for the PD-inducing effects of MPTP. Figure 1A shows the density derived from Western blot bands for striatal TH in groups of C57BL/J6 mice. The representative expression is shown as two sample bands from each treatment group, corresponding to the columns on the bar graph. The mice in the PBS and MPTP groups were treated sub-chronically with either L-DOPA or with PBS, to produce 4

groups: (1) *PBS+PBS*, (2) *MPTP+PBS*, (3) *PBS+L-DOPA* and (4) *MPTP+L-DOPA*. The results in figure 1A show that the MPTP treatment depleted TH by 60%, (2nd column, *MPTP+PBS*) as compared to the *PBS+PBS* group (1st column). Interestingly, the administration of L-DOPA to the PBS control mice decreased the expression of TH by 40% (3rd column). When L-DOPA was administered to the MPTP group, with an already depleted TH pool, L-DOPA did not show the reductive effect seen in the PBS control (4th column). This suggests a lack of homeostatic property for the MPTP parkinsonian striatal TH system, since a down-regulation of TH is a reasonable expectation to be caused by L-DOPA, on the basis that the administered L-DOPA may cause end-product inhibition of TH. It was somewhat unexpected that treatment of animals with L-DOPA alone caused a decrease in the expression of TH, but this same treatment did not lead to any loss in DA metabolites. While the decrease in TH can be associated with toxicity, this loss can be also attributed to a “loss of use” of the TH enzyme, because when L-DOPA is administered in large doses, TH is bypassed and AAAD catalyzes the production of dopamine. So, the high levels of exogenous L-DOPA could cause feed-back inhibition of TH (Molinoff P.B. et al. 1971) and also serve to maintain the levels of DA and its metabolites as the intermediary substrate between tyrosine and DA

Figure 1B highlights the effects of MPTP and L-DOPA treatments on the mice striatal DA levels. MPTP depleted DA by 80% (2nd vs 1st column), which is in conformity with the PD-like toxic effects of MPTP. It was of interest that rather than an increase in DA, the MPTP animals that received L-DOPA, showed a reduction in DA by about 40 %, when compared to the *MPTP+PBS* group (Figure 1b). This is relevant to the fact that at 24 hours after L-DOPA, when the tissue levels of L-DOPA are back to the non-treatment low level, the enzymes that synthesize DA, TH and AAAD, are also correspondingly down-regulated by L-DOPA (King and Charlton 2008), so DA levels ought to be low.

Striatal AAAD expression and its activity in the MPTP model versus PBS control

It was also of interest to examine the regulatory effects of MPTP and L-DOPA on the expression and activity of AAAD following the 24 hr period for the elimination of exogenous L-DOPA. Figure 2A shows the expression of AAAD and figure 2B shows the activity of AAAD in the striatum. Figure 2A reveals a slightly higher, but not significantly different, level of expression of striatal AAAD in the MPTP (only)-treated animals relative to control animals. AAAD expression is not altered compared to control (PBS-treated) animals upon L-DOPA treatment unless L-DOPA is administered to MPTP treated animals. These findings suggest that L-DOPA treatment in the context of DA neurons leads to a decrease in AAAD expression and could account, at least in part, to the inability of the MPTP injured neurons to replenish AAAD. These observations could also provide insight into the toxic side effects and waning efficacy of L-DOPA after prolonged administration in PD patients. It is of interest that the administration of L-DOPA to the MPTP PD animal resulted in a dramatic reduction of 50% in the expression of AAAD (figure 2A, 4th vs. 2nd column). So, the DA enhancing precursor molecule, L-DOPA, decreases the steady-state expression of striatal AAAD in the MPTP damaged catecholamine system. Taken together, a dynamic counter-response to MPTP and to L-DOPA, agents that manipulate the dopaminergic system, may occur.

The enzymatic activity of AAAD helps to evaluate the state of the enzyme. The study shows that MPTP treatment that generates the PD model decreased the activity of AAAD by 32.5% (figure 2B, 2nd vs. 1st columns). It is of interest to note that the reduction of AAAD activity by MPTP is opposite to the effect of increasing the expression of AAAD, caused by MPTP (figure 2A, 2nd column), so MPTP, that seems to increase the expression of the enzyme may have impaired the catalytic ability of AAAD to convert L-DOPA to DA, thus a reduction in its activity (figure 2B, 2nd column), but the counter response may be an increase in the

expression of the enzyme as shown in figure 2A, 2nd column. L-DOPA treatment did not affect the steady-state activity of AAAD in the normal PBS mice, but the administration of L-DOPA to the MPTP-treated animals caused a further slight reduction in the activity of AAAD (figure 2B, 4th column). In summary MPTP increased the steady-state expression of AAAD, but decreased the steady-state activity of the enzyme, and L-DOPA decreased both the expression and the activity of AAAD in the MPTP-treated mice (although the activity was not significantly affected). The down-regulation of the expression of AAAD in the MPTP PD model by L-DOPA at 24 hr after L-DOPA is of importance, because it may help to explain that tolerance or the loss of efficacy of L-DOPA that occurs in PD patients.

L-DOPA treatment down-regulates the native supply of DA and its metabolites

The results show that DA, DOPAC and HVA were reduced by 72.3, 49.1 and 13.1% in the MPTP+PBS group as compared to the PBS+PBS control. The calculation of DOPAC+HVA/DA shows that an increase in turnover of DA occurs; 0.87 for the PBS+PBS control vs. 2.4 for the MPTP+PBS group. DA levels did not appreciably change following the L-DOPA treatment (PBS+L-DOPA group) as compared to the PBS+PBS group, but DOPAC was reduced by 22% and HVA by 15%. This shows that there was a slight decrease in the turnover of DA (DOPAC+HVA/DA). The L-DOPA treatment to the MPTP mice caused marked losses in DA, DOPAC and HVA by 81.65%, 81.1% and 97.8% when compared to the PBS+PBS control. When the MPTP+PBS group is used as the comparison for the MPTP-L-DOPA group, the percentage differences in reduction caused by L-DOPA are 10.3%, 32.1% and 84.8% for DA, DOPAC and HVA respectively. The measures were made at 24 hr after L-DOPA, when externally derived L-DOPA was not contributing to the levels of DA. It can be concluded, therefore, that L-DOPA treatment in a PD model reduced the capacity of the native dopaminergic system to synthesize DA. This is probably due to the down-regulation of the catecholamine synthesis capacity by the infusion of exogenous L-DOPA and a failure in recovery within 24 hrs post-L-DOPA.

Tracking the course of DA and AAAD post-L-DOPA cessation

DA is the product of L-DOPA and AAAD is the enzyme that catalyzes the decarboxylation of L-DOPA to produce DA, so we tracked the levels of DA and the activity of AAAD following L-DOPA. We accessed the time-course for the changes in the levels of DA and the activity of AAAD and the relationship that exists between DA and AAAD. The MPTP treated mice were primed with L-DOPA, administered twice a day for 14 days. Again, the aim is to understand the time course of the effects caused by the sub-chronic administration of L-DOPA on the DA levels and the activity of AAAD. The DA levels and the activity of AAAD were determined at 0, 4, 24 and 36 hours following the 24 hr withdrawal time. Figure 3 shows that at 28 hr post L-DOPA, AAAD activity showed further decline, below the 24 hr activity and troughed at 48 hrs post-L-DOPA and recovered to the 24 hr post-L-DOPA level of activity at 60 hr. So the changes present a concave pattern (figure 3). DA levels were increased at the 28 hr period, peaked at 48 hrs post-L-DOPA and its level declined at 60 hrs to an average amount that was about twice the level at 24 hrs post L-DOPA. The curve for DA presents a convex pattern (figure 3). It was also shown that the pattern of the curve for L-DOPA over the same time period was convex (data not shown). Parallel results were found in a closer time-course study (figure 4) which shows that immediately after the cessation of L-DOPA the same inverse relationship is displayed between the activity of AAAD and DA levels, but at higher levels. The relationship of the activities of AAAD and the levels of DA show that when the level of DA is low, the activity of AAAD is high and vice-versa, so the regulatory system may up-regulate the activity of AAAD to replenish the low DA and when DA accumulation is increased the activity of AAAD is down-regulated. This relationship may serve to restore DA when the levels of the

neurotransmitter are low, and can be viewed as an almost inverse relationship between the activity of striatal AAAD and the levels of striatal DA.

DISCUSSION

The aim of the present study was to determine the effect of L-DOPA treatment on the expression and activity of AAAD using the MPTP rodent PD model. Most of the analysis and measurements were made at 24 hrs following the final dose of L-DOPA so as to eliminate the acute effects of high tissue levels of the exogenous L-DOPA. We also analyzed tissue immediately after the last dose of L-DOPA to examine the acute effects of exogenous L-DOPA on DA levels and AAAD activity.

First, the outcome confirms previous studies showing that MPTP reduced TH as well as DA. MPTP exposure in C57 mice has been shown in numerous studies to cause striatal DA depletion (Corsin, Kopin et al. 1987, Nishi, Narabayashi et al. 1991, Sundstrom, Archer et al. 1990, Fredriksson, Archer et al. 1990, Willis and Donnan 1987, Bing, Stone et al. 1994) and neuronal cell death in the substantia nigra (Ricaurte, Langson et al. 1987, Jackson-Lewis V, Przedborkis S. et al. 1995, Tatton and Kish 1997) similar to what is seen in humans (Forno 1966, Alvord 1968). Since it is well known that MPTP causes neuronal degeneration, a condition that should also reduce AAAD, the slight elevation of the expression of AAAD (Fig 2A, 2nd column) and reduction in its activity (Fig 2B, 2nd column) seen following MPTP may be interpreted to mean that a compensatory increase in the synthesis of AAAD with lower potency was caused by MPTP. However, since the Western blot determination of AAAD could not distinguish the DA derived AAAD from other biogenic amines, such as serotonin that also produce AAAD, other biogenic amine-containing neurons that are not as sensitive to MPTP may show a compensatory increase in AAAD that was measured in our assay.

L-DOPA has no effect on AAAD in the control animals but in the MPTP model L-DOPA reduces both the protein expression and the activity of AAAD (Fig 2A and 2B, columns 2 vs. 4). So, MPTP treatment may render the enzyme more responsive to L-DOPA. Whether that responsiveness is due to cell damage caused by MPTP is not known, but this type of pathological responsiveness to L-DOPA may also contribute to its level of efficacy for PD, a finding that may help in resolving the therapeutic efficacy as well as the side-effects seen following L-DOPA therapy.

The down-regulation of the expression and to some extent the activity of AAAD by L-DOPA may help to emphasize the utility of AAAD as the limiting step in the catecholamine pathway during L-DOPA therapy of PD as noted by Hadjiconstantiou and Neff (2008). Moreover, the mystery underlying the mechanisms of the failure of the oral L-DOPA treatment (Colosimo and De Michele 1999) and the requirements for increasing the dosage of L-DOPA to achieve a better control of PD symptoms may also be related to the down-regulation of the activity of AAAD by L-DOPA. Since, in the presence of a down-regulated AAAD, the robust increases in DA will not be realized. More importantly, the down-regulation of AAAD may also help to explain other side effects of L-DOPA. For example, in the presence of a down-regulated AAAD, L-DOPA will accumulate and its metabolism may be shunted to the production of metabolites, such as 3-O-methyl-DOPA, that is well known to antagonize the efficacy of L-DOPA. (Lee et al. 2008).

It is of importance to note that at 24 hr after the last sub-chronic dose of L-DOPA in the MPTP model, the steady state levels of DA, HVA and DOPAC were appreciably lower in the MPTP group that received L-DOPA than in the matching MPTP group that received PBS injections (table 1). This means that L-DOPA decreased the native synthesis and

metabolic machinery for the catecholamine. This was appreciably more severe in the MPTP mouse model than in the PBS group, imparting the assumption that the affected striatum of the MPTP model is unstable and/or insufficient; conclusion supported by studies in unilateral 6-hydroxydopamine-lesioned rats (Buck and Ferger 2008). The studies of Buck and Ferger (2008) used rats that were unilaterally lesioned with the loss of striatal dopaminergic innervation. In their study the non-lesioned side was capable of maintaining the homeostasis of DA levels when L-DOPA was administered, but maintaining homeostatic levels of DA was not possible by the lesioned side. So, it turns out that the dopaminergic system of the MPTP mice that were treated with L-DOPA was in a worse state of reduction as compared to similar MPTP mice that were treated with PBS. The study also suggests that the steady-state levels of DA show a close inverse relationship to the activity of AAAD (Figures 3 and 4), so the regulatory machinery in the brain may up-regulate the activity of AAAD to replenish the low DA and when DA accumulation is increased the activity of AAAD is down-regulated. This relationship may serve to restore DA when the level of the neurotransmitter is low.

Though down-regulation of AAAD activity in MPTP-treated animals would be expected to occur as DA neurons are lost, the present data suggest that administration of L-DOPA and resultant increases in DA synthesis may support additional regulatory phenomena that reduce AAAD activity, namely direct negative feedback effects of L-DOPA-supported DA synthesis on AAAD activity. It is well known that while L-DOPA is very beneficial in correcting the PD symptoms, it is bothered with serious side effects, including a loss of efficacy, so that some clinicians avoid L-DOPA therapy as long as possible in an effort to delay the onset of L-DOPA complications (Albin and Frey 2003 Albin et al. 2006). The down regulation of AAAD may help to explain the loss of efficacy that occurs following L-DOPA therapy.

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1B.

Figure 1. Tyrosine hydroxylase expression and DA levels in MPTP mouse model treated with L-DOPA or PBS

Figure 1 A shows columns (lower portion) depicting the quantitative values of the protein bands (upper portion) derived from Western blot determination of striatal tyrosine hydroxylase (TH) in PBS control and MPTP treated (30 mg/kg once a day for 7 days) mice that received either sub-chronic L-DOPA (100 mg/kg twice a day for 14 days) or PBS. The mice were sacrificed 24 hrs after the last dosage of L-DOPA. The results show that MPTP drastically reduced TH by 60% in the PBS animals (2nd column). L-DOPA also reduced TH in the PBS mice (3rd column), but show not additive effect in the MPTP mice (4th column). Figure 1B highlights the changes in DA in the same groups. Again, MPTP depleted DA (2nd column), DA remains stable in the PBS mice treated with L-DOPA (3rd column) and L-DOPA reduced DA by 20% in the MPTP mice. (4th column). The data means that sub-chronic L-DOPA decreased the DA synthesis capability in the striatum of MPTP PD mouse model. The determinations were made 24 hrs after the last dosage of L-DOPA, and are regarded as the 'steady-state' levels, due to plastic changes from enduring the high tissue level of L-DOPA. Each data point was obtained from 6–8 animals and presented as the mean $\pm$ S.E.M, and evaluated using ANOVA with a Tukey post test. Astericks represent significance with $p < 0.05$ and compared with *PBS+PBS*.

Figure 2. Aromatic l-amino acid decarboxylase (AAAD) expression and activity in MPTP mouse model treated with L-DOPA or PBS

Figure 2 A shows the quantitative values of protein bands (upper portion) derived from Western blot determination of AAAD in PBS and MPTP treated (30 mg/kg once/day for 7 days) mice that received L-DOPA (100 mg/kg twice/dy for 14 days) or PBS and sacrificed 24 hrs after the last dosage of L-DOPA. MPTP showed a slight increase in AAAD (1st vs. 2nd columns), but L-DOPA showed no effect as compared to the PBS+PBS mice. In the MPTP PD model, however, L-DOPA reduced the expression of striatal AAAD (2nd vs. 4th column). Figure 2B highlights the changes in AAAD activity in the same groups. MPTP caused 29% reduction in the activity of AAAD (2nd vs. 1st column). L-DOPA shows no effect in the PBS treated mice, but it reduced AAAD activity in the MPTP mice by 14%. This was not significantly different at the 5% level of probability (4th column), the caused an additive 53% reduction when the MPTP and the L-DOPA effects are noted. So, L-DOPA down-regulates the synthesis machinery for DA in the MPTP model. The determinations were made 24 hrs after the last dosage of L-DOPA, and are regarded as the ‘steady-state’ levels, due to plastic changes from the sub-chronic high tissue level of L-DOPA. Each data point was obtained from 6–8 animals and presented as the mean ± S.E.M, and evaluated using ANOVA with a Tukey post test. Astericks represent significance with $p < 0.05$ and compared with *PBS+PBS*.

AAAD Activity vs. Dopamine Levels

Figure 3. Tracking the course of DA and AAAD beyond the 24 hr post-L-DOPA time
Time-course relationship of the activity of LAAD and the levels of DA in the MPTP+L-DOPA treated mice allows us to estimate the regulatory relationship that exists between the constituents. The AAAD activity and DA levels of a group of MPTP lesioned L-DOPA treated mice were measured starting at 24 hrs after the last injection of L-DOPA and at 4, 24 and 36 hours intervals, or measurements were made at 24, 28, 48 and 60 hrs after the cessation of L-DOPA treatment. The plot of the concentration of DA and the activity of AAAD show a concave curve for AAAD (solid line) and a convex curve for DA (broken line). This relationship suggests that when DA level is low the regulatory command increased the activity of AAAD to restore the levels of DA and when DA level is high the command for AAAD down-regulates its activity so as not to produce too high a level of DA. Data are mean $\pm$ S.E.M. values for 8 mice per group.

AAAD Activity vs. Dopamine Levels

Figure 4. Tracking the course of DA and AAAD immediately after L-DOPA administration

The AAAD activity and DA levels of a group of MPTP lesioned L-DOPA treated mice were measured starting 0 min after the last injection of L-DOPA and at 30, 60, and 90 min intervals after the cessation of L-DOPA treatment. The plot of the concentration of DA and the activity of AAAD show an increase for AAAD (solid line) and a decrease for DA (broken line). This relationship suggests an inverse regulatory mechanism between DA and AAAD. Data are mean $\pm$ S.E.M. values for 5–7 mice per group.

Table 1
L-DOPA treatments down-regulate the native synthesis capability for dopamine and its metabolites in the MPTP PD mouse model

The table shows the levels and the percent concentrations of DA, DOPAC and HVA determined in groups of MPTP or PBS mice treated with L-DOPA or PBS to give: PBS+PBS; MPTP+PBS; PBS+L-DOPA and MPTP +L-DOPA groups. L-DOPA (100 mg/kg i.p) or PBS was given sub-chronically twice/dy for 14 days. Measurements were made 24 hrs after the last dosage of L-DOPA to obtain the 'steady-state' levels of DA, DOPAC and HVA. The effects are seen as plastic changes and not directly caused by high tissue levels of L-DOPA. The table shows that MPTP reduced DA, DOPAC and HVA, by 71.3, 49.1 and 13.1% as compared to the PBS control. L-DOPA had a slight effect in the PBS control mice reducing DA, DOPAC and HVA by 4, 17, and 26%, and the exposure of the MPTP mice to L-DOPA cause a significant reduction of DA, DOPAC and HVA of 81.6, 81.1 and 97.8%. So, the data show that sub-chronic L-DOPA reduced the metabolic capability of DA and its metabolites in the MPTP PD mouse model. The turnover of DA was increased to 2.4 in the MPTP mice, but it was decreased in the MPTP+L-DOPA mice to 0.28. Each group contains 6–8 mice. * $p < 0.05$.

Treatment	Dopamine %	DOPAC %	HVA %	DOPAC+HVA/Dopamine
PBS+PBS	100	100	100	0.87
PBS+L-DOPA	95.58±2.1	83.01±6.1	73.91±3.1	0.69
MPTP+PBS	28.68±1.1 *	50.94±5.2 *	86.96±2.7	2.4 *
MPTP +L-DOPA	18.38±1.7 *	18.87±3.4 *	2.17±2.4 *	0.28 *

* $p < 0.05$ compared with the PBS+PBS or MPTP+PBS