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L-DOPA-induced 6-hydroxydopamine production in the striata of rodents is sensitive to the degree of denervation

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ABSTRACT

We tested the hypothesis that long-term L-DOPA treatment in Parkinson's disease (PD) would result in increased production of the dopamine oxidation product, 6-hydroxydopamine (6-OHDA) in the striatum. We administered L-DOPA (250 mg/kg) by gavation for 3 days following 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP; 30 mg/kg, i.p., twice, 16 h apart) in mice or for 30 days in rats which received 1-methyl-4-phenylpyridinium ion (MPP^+; 16 nmol/1 μl) unilaterally in median forebrain bundle, and measured 6-OHDA in the striatum using a sensitive HPLC-electrochemical detection procedure. While the contralateral innervated striatum of rats showed no difference, a significant increase in 6-OHDA level in the denervated (>85% dopamine depletion) ipsilateral striatum was observed. Partial nigrostriatal denervation with a lower dose of MPP^+ (8 nmol/1 μl) in rats or following sub-acute MPTP treatment in mice failed to cause any significant change in 6-OHDA level following several doses of L-DOPA administration. Since a single dose of MPTP (30 mg/kg, i.p.) or L-deprenyl (0.25 mg/kg, i.p.) in L-DOPA primed (250 mg/kg daily for 7 days) mice caused significant increase in 6-OHDA in the striatum, augmentation of reactive oxygen species production concomitant to excessive dopamine concentration in this region is proposed to be the basis of this effect. These results suggest creation of potential pro-toxic environment in the brain due to the long-term administration of L-DOPA, which may get further sensitized by the treatment of monoamine oxidase inhibitors.

1. Introduction

Human brain biopsies (Curtius et al., 1974) and urine samples (Andrew et al., 1993) of Parkinson's disease (PD) patients have been reported to contain the potent dopaminergic neurotoxin, 6-hydroxydopamine (6-OHDA), a dopamine (DA) oxidation product. Methamphetamine administration has been reported to produce (Seiden and Vosmer, 1984; Liao et al., 2003) this molecule endogenously in the brains of rats and mice, which has been refuted by others (Rollema et al., 1986; Karoum et al., 1993). In vitro studies have demonstrated the generation of 2-hydroxydopamine, 5-hydroxydopamine and 6-OHDA in the past (Slivka and Cohen, 1985; Napolitano et al., 1999) and more recently by Maharaj et al. (2005) and by us (Borah and Mohanakumar, 2009a,b), from DA in the presence of ascorbic acid and iron. In these two latter studies, significant production of 6-OHDA in vivo in the striatum of rodents has been demonstrated as a result of chronic treatment of L-3,4-dihydroxyphenylalanine (L-DOPA) in animals that received intrastriatal infusion of ferrous sulfate (Maharaj et al., 2005) or systemic injection of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Borah and Mohanakumar, 2009b). Significantly, Andrew et al. (1993) reported a significant increase in 6-OHDA levels in urine of L-DOPA treated PD patients as compared with patients without L-DOPA treatment or normal subjects.

MPTP is known to cause PD in humans and subhuman primates (Langston et al., 1983; Burns et al., 1983), and to produce biochemical syndromes of parkinsonism in rodents (Heikkila et al., 1984; Chiueh et al., 1993; Mitra et al., 1992). It is now an accepted fact that MPTP gets oxidized in the brain by monoamine oxidase-B (MAO-B; EC 1.4.3.4) to produce 1-methyl-4-phenyl pyridinium ion (MPP^+) (Heikkila et al., 1984; Cohen et al., 1984) which is selectively taken up into the dopaminergic neurons via dopamine transporters (Gainetdinov et al., 1997) where it gets accumulated in the mitochondria (Ramsay and Singer, 1986) causing excessive reactive oxygen species generation (Chiueh et al., 1993; Thomas et al., 2000; Thomas and Mohanakumar, 2004), and severe oxidative stress (Chiueh et al., 1993; Mohanakumar and Muralikrishnan, 2000; Banerjee et al., 2008) culminating in the degeneration of these neurons in the brain. Most of these events are also suggested to occur in PD pathogenesis (Schapira, 2008). Therefore MPTP or its active neurotoxic metabolite, MPP^+ is

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regarded as one of the most preferable candidates for producing cellular (Leonardi and Mytilineou, 1998; Collier et al., 2003) and animal models (Beal, 2001; Jenner, 2008) of parkinsonism, and by dint of which, much information has come to light on the pathological basis of this disease (Gupta et al., 2008; Schapira, 2009).

L-DOPA, the precursor amino acid of dopamine, is the treatment of choice even now for PD in humans (Schapira et al., 2009), despite severe side effects following prolonged treatment (Cenci, 2007; Fox et al., 2008). The potential of this precursor molecule to produce toxic reactive oxygen species (Jenner and Brin, 1998), and detrimental oxidation products of dopamine (Graham, 1978; Maharaj et al., 2005), especially when made available in excess in the cytosolic fractions (Mosharov et al., 2009) has been known in literature, and is still debated. Recent studies from our laboratory have demonstrated production of dopamine in neurons other than dopaminergic in nature following chronic treatment of L-DOPA suggested this as a basis for the observed unpredictable outcome of such treatment in PD, and the on–off phenomenon observed in these patients (Borah and Mohanakumar, 2007).

L-Deprenyl (Selegiline), a potent monoamine oxidase-B (MAO-B) inhibitor (Knoll, 1993; Youdim and Bakhle, 2006), with proven antiparkinsonian activity, is reported to possess significant neuroprotective effects by inhibiting reactive oxygen species, especially hydroxyl radical ($^{\bullet}OH$) generation in the brain (Chiueh et al., 1994; Thomas et al., 1997; Muralikrishnan et al., 2003; Saravanan et al., 2006) and by up-regulating antioxidant enzymes, such as catalase and superoxide dismutase (Carrillo et al., 1992; Muralikrishnan et al., 2003; Saravanan et al., 2006). It has been shown to attenuate MPTP-induced neurotoxicity in rodents and protect against $^{\bullet}OH$ mediated neurodegeneration both in vitro and in vivo (Mohanakumar and Muralikrishnan, 2000; Leret et al., 2002; Muralikrishnan et al., 2003; Saravanan et al., 2006).

Based on these prevailing state of the art in literature, it was thought prudent to assess the levels of a potentially neurotoxic DA-oxidation product, 6-OHDA in the striatum of MPTP and MPP^+ models of parkinsonism respectively in mice and rats that received prolonged L-DOPA treatment. In the present study, we also investigated 6-OHDA production in vitro in ferrous-ascorbate-dopamine (FAD) system in test tubes, and the effects of L-deprenyl thereof.

2. Materials and methods

2.1. Chemicals

L-DOPA tablets (Syndopa) were purchased from Sun Pharmaceutical Industries Ltd. (Baroda, India) containing 10% of carbidopa, and solubilized in water. MPTP HCl, MPP^+ iodide, ascorbic acid, 6-OHDA, DA HCl, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), L-DOPA, L-deprenyl, 1-heptane sulfonic acid sodium salt and ethylenediaminetetraacetic acid disodium salt (EDTA) were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). Octane-1-sulfonic acid, ferrous sulfate, perchloric acid and acetonitrile were obtained from Merck, Mumbai, India. Chloral hydrate was purchased from Fluka (Buchs, Switzerland). Sodium phosphate monobasic and citric acid were purchased from MP Boimedicals Inc. (Illkirch, France).

2.2. Animals

Male adult Balb/C mice (20–25 g) and Sprague–Dawley rats (250–300 g) were used in the present study. The animals were maintained under standard conditions of 12 h light/dark cycles, 24 ± 2 °C temperature and $60 \pm 5\%$ humidity. They were provided with food and water *ad libitum*. The experimental protocols met the National Guidelines on the "Proper Care and Use of Animals in Laboratory Research" (INSA, 2000) and were approved by the Animal Ethics Committee of the Institute.

2.3. Drug treatment

2.3.1. Rat brain stereotaxic surgery

Rats were anaesthetized with chloral hydrate (400 mg/kg, i.p.). The animals were held over a stereotaxic frame (Steolting, Wood Dale, IL, USA) with incisor bar kept at 3.5 mm below the interaural line. MPP^+ (8 or 16 nmol/1 μl) was dissolved in saline and was infused into the right median forebrain bundle (MFB) at the flow rate of 0.2 μl/min employing a micro-infusion apparatus consisting of Worker bee and Syringe Pump (Bioanalytical Systems, West Lafayette, IN, USA). After infusion the probe was kept for further 5 min to allow proper diffusion of the toxin and then slowly retracted. The coordinates used for MFB were anterio-posterior: 0.28, lateral: 0.15 and dorso-ventral: 0.82 from the Bregma point (Paxinos and Watson, 1998).

2.3.2. L-DOPA administration in hemiparkinsonian rats

MPP^+ (8 nmol and 16 nmol/1 μl) infused rats (in MFB) were administered with L-DOPA (250 mg/kg) for 30 days along with carbidopa (25 mg/kg) daily from the 7th day post infusion, and were sacrificed 2 h after the last dose of the drug. For each group the striatal 6-OHDA level was analyzed. Striatal monoamine levels were analyzed only in L-DOPA naive MPP^+ administered animals.

2.3.3. L-DOPA administration in MPTP-induced parkinsonisonian mice

Mice were treated with MPTP 16 h apart, at the dose of 30 mg/kg i.p. as has been extensively demonstrated in our laboratory that would result in 50–60% striatal DA depletion (Muralikrishnan and Mohanakumar, 1998; Thomas et al., 2000; Thomas and Mohanakumar, 2004; Muralikrishnan et al., 2003). Animals received the first injection at 1700 h and second at 0900 h the next day. The animals were administered L-DOPA containing carbidopa (250 and 25 mg/kg respectively; daily) for 3 consecutive days and sacrificed at 2 h after the 3rd dose of the drug. For each group the striatal 6-OHDA level was analyzed. Striatal monoamine levels were analyzed only in L-DOPA naive MPTP administered animals.

2.3.4. Acute administration of MPTP in L-DOPA primed mice

Two groups of mice were treated with L-DOPA (250 mg/kg daily) along with carbidopa (25 mg/kg) for 7 days and another two groups received vehicle for 7 days. A single dose of MPTP (30 mg/kg, i.p.) was administered in the first group of animals that received L-DOPA or vehicle for 7 days. Ninety minutes following the last injection of MPTP or 2 h following L-DOPA or vehicle, all the animals were sacrificed and assayed for 6-OHDA in their striatum.

2.3.5. Acute administration of MPTP in L-DOPA and L-deprenyl pretreated mice

Three groups of mice received L-deprenyl (0.25 mg/kg) dissolved in saline daily for 7 days, 30 min prior to L-DOPA or vehicle administration. The first set of mice was given a single dose of MPTP (30 mg/kg, i.p.) 90 min before sacrifice. For each group the striatal 6-OHDA levels were analyzed at 2 h after the last dose of L-DOPA or vehicle.

2.4. Analysis of 6-OHDA by HPLC-ECD

Whole brains from mice were dissected out, rinsed in chilled normal saline, blotted dry on ash-free filter paper and the striata were dissected out. The tissue was weighed and sonicated in 1 volume of chilled perchloric acid (0.4 M) containing ascorbic acid (1 mM). It was kept on ice for 30 min and centrifuged at $18{,}000 \times g$ for 5 min, supernatant was collected and 10 μl was injected into the HPLC. The mobile phase consisted of 25 mM citric acid, 125 mM sodium phosphate, 100 mg/l EDTA and 30 mg/l octane sulfonic acid and pH adjusted to 2.5 with 85% ortho-phosphoric acid (Maharaj et al., 2005). Flow rate was maintained at 0.7 ml/min and the electrochemical detection was performed at +200 mV. The HPLC system consisted of an isocratic pump (Bioanalytical Systems, West Lafayette, IN, USA), an amperometric detector (Epsilon, Bioanalytical Systems) and C18, ion pair, analytical column (4.6 mm × 250 mm; Ultrasphere IP; Beckman, Fullerton, CA, USA), with particle size of 5 μm and pore size of 80 Å.

2.5. HPLC analysis of biogenic amines

Brains were removed from the calvarium and striata were processed for the analyses of the biogenic amine neurotransmitters and their metabolites employing an HPLC-electrochemical detection procedure (Muralikrishnan and Mohanakumar, 1998; Borah and Mohanakumar, 2007). The tissues were sonicated in ice-cold 0.1 M perchloric acid containing 0.01% EDTA. The supernatant collected after centrifugation at $18{,}000 \times g$ for 5 min was injected (10 μl) into the HPLC system. The flow rate was maintained at 0.7 ml/min and the electrochemical detection was performed at +740 mV. The composition of the mobile phase was 8.65 mM heptane sulfonic acid, 0.27 mM EDTA, 13% acetonitrile, 0.43% triethylamine and 0.32% ortho-phosphoric acid. The HPLC system is the same as described earlier.

2.6. Generation of 6-OHDA from FAD system

We used the FAD system as described by Slivka and Cohen (1985), with and without L-deprenyl at increasing concentrations (0.01, 0.1, 1 mM). DA was always added in the last. The reaction mixture was incubated at 37 °C in dark for 120 min. Aliquotes (100 μl) were removed and reaction was stopped by adding 900 μl of ice-cold 0.4 M perchloric acid containing 1.0 mM ascorbate. Ten microliter of this sample was injected into HPLC system for 6-OHDA analysis. Final results are expressed as pmol/ml. The FAD system incubated for 120 min with 1 mM DA or