CEP-1347/KT-7515, an Inhibitor of c-jun N-Terminal Kinase Activation, Attenuates the 1-Methyl-4-Phenyl Tetrahydropyridine-Mediated Loss of Nigrostriatal Dopaminergic Neurons In Vivo

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ABSTRACT
We have identified a bis-ethylthiomethyl analog of K-252a, CEP-1347/KT-7515, that promotes neuronal survival in culture and in vivo. The neuronal survival properties of CEP-1347/KT-7515 may be related to its ability to inhibit the activation of c-jun N-terminal kinase, a key kinase in some forms of stress-induced neuronal death and perhaps apoptosis. There is evidence that the selective nigrostriatal dopaminergic neurotoxin, MPTP, produces neuronal apoptosis in culture and in adult mice. Thus, our studies were designed to determine if CEP-1347/KT-7515 could protect dopaminergic neurons from MPTP-mediated neurotoxicity. CEP-1347/KT-7515 was assessed for neuroprotective activity in a low dose MPTP model (20 mg/kg) where there was a 50% loss of striatal dopaminergic terminals in the absence of substantia nigra neuronal loss, and a high dose (40 mg/kg) MPTP model where there was a complete loss of dopaminergic terminals and 80% loss of dopaminergic cell bodies. In the low dose MPTP model, CEP-1347/KT-7515 (0.3 mg/kg/day) attenuated the MPTP-mediated loss of striatal dopaminergic terminals by 50%. In the high dose model, CEP-1347/KT-7515 ameliorated the loss of dopaminergic cell bodies by 50% and partially preserved striatal dopaminergic terminals. CEP-1347/KT-7515 did not inhibit monoamine oxidase B or the dopamine transporter, suggesting that the neuroprotective effects of CEP-1347/KT-7515 occur downstream of the metabolic conversion of MPTP to MPP+ and accumulation of MPP+ into dopaminergic neurons. These data implicate a c-jun N-terminal kinase signaling system in MPTP-mediated dopaminergic degeneration and suggest that CEP-1347/KT-7515 may have potential as a treatment for Parkinson's disease.

Parkinson's disease is a progressive degenerative disorder involving a relatively specific neurodegeneration of the nigrostriatal dopaminergic tract (for review see Agid, 1991). Several aspects of this neurodegenerative disease can be replicated in mice and in primates by administration of 1-methyl-4-phenyl tetrahydropyridine (MPTP), a selective nigrostriatal dopaminergic neurotoxin. In mice, systemic administration of MPTP produces a loss of striatal dopamine (DA) nerve terminal markers and, at higher doses, dopaminergic neuronal death in the substantia nigra (Heikkila et al., 1984a,b; Tipton et al., 1993; Jackson-Lewis et al., 1995).

The dopaminergic neurotoxicity of MPTP is dependent on: 1) the monoamine oxidase B (MAO-B)-mediated 2-electron oxidation of MPTP to MPP+ in the central nervous system (Heikkila et al., 1984); 2) active uptake of MPP+ into dopaminergic neurons via the DA transporter (Javitch et al., 1985; Saporito et al., 1992); 3) accumulation of MPP+ in mitochondria (Tipton et al., 1993); and 4) inhibition of complex I of the electron transport chain (Nicklas et al., 1985; Kindt et al., 1987; Saporito et al., 1992). Uncoupling of mitochondrial respiration produces a number of secondary events including loss of neuronal ATP, elevations in intraneuronal calcium levels and increased oxidative stress (Chan et al., 1991; Schulz et al., 1995). More recently, MPTP was found to produce morphological characteristics of apoptosis in neurons. For example, addition of MPP+ to PC12 cells, cerebellar granule cells, and primary cultured mesencephalic neurons elicits nuclear chromatin condensation, membrane blebbing, and DNA laddering (DiPasquale et al., 1991; Hartley et al., 1994; Mochizuki et al., 1994). Furthermore, MPTP administration to mice increases the number of neurons with double-strand DNA breaks and chromatin clumping, suggesting that at least some of these neurons are undergoing apoptotic death (Tatton and Kish, 1997).

ABBREVIATIONS: DA, dopamine; DOPAC, dihydroxyphenylacetic acid; GDNF, glial derived neurotrophic factor; HVA, homovanillic acid; Jnk, c-jun N-terminal kinase; MPP+, 1-methyl phenylpyridine; MPTP, 1-methyl-4-phenyl tetrahydropyridine; MAO, monoamine oxidase; TH, tyrosine hydroxylase; PBS, phosphate-buffered saline.
We have identified a novel bis-ethylthiomethyl analog of the indolocarbazole K-252a, CEP-1347/KT-7515, that promotes survival and/or increases functional parameters in several neuronal systems (for structure see Kaneko et al., 1997). For example, CEP-1347/KT-7515 promotes survival of chick dorsal root ganglia and increases choline acetyltransferase enzyme activity in neurons derived from embryonic rat basal forebrain (Kaneko et al., 1997). Interestingly, CEP-1347/KT-7515 prevents the programmed cell death of developing motoneurons of the spinal nucleus bulbocavernous after testosterone withdrawal and of developing motor neurons in the chick embryo (Glicksmans et al., 1998), suggesting that the neuronal survival properties of CEP-1347/KT-7515 may be related to its ability to inhibit a signal leading to apoptosis. In fact, the neuronal survival effects of CEP-1347/KT-7515 in cultured embryonic motoneurons correlates with its ability to inhibit activation of c-Jun N-terminal kinase (Jnk), a key kinase in some forms of stress-induced neuronal death and perhaps apoptosis (Ham et al., 1995; Xia et al., 1995; Maroney et al., 1998).

Because MPTP can produce characteristics of apoptosis in cultured neurons and in vivo, CEP-1347/KT-7515 was assessed for its ability to prevent MPTP-mediated nigrostriatal dopaminergic damage. CEP-1347/KT-7515 was assessed under two conditions of MPTP administration: 1) a low dose MPTP paradigm in which only dopaminergic terminals in the striatum were lost and 2) a high dose MPTP dosing paradigm in which terminals and cell bodies were lost. The data from these studies demonstrate that CEP-1347/KT-7515 prevents MPTP-mediated nigrostriatal dopaminergic degeneration in vivo, implicating the Jnk signaling pathway in MPTP-mediated neurodegeneration and suggest that this molecule may be a potential treatment for slowing or halting the progression of Parkinson’s disease.

**Materials and Methods**

**Animals, Neurotoxin, and Drug Treatment.** All procedures using animals were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animals Care and Use Committee. Male C57 black mice weighing between 20 and 25 g (Charles River) were used for all experiments. Animals were maintained on a standard 12 h light/dark cycle and were allowed food and water ad libitum.

**MPTP and CEP-1347/KT-7515 Treatments.** MPTP (Research Biochemicals International, Natick, MA) was dissolved in PBS at concentrations of 2 or 4 mg/ml. Mice received a single s.c. injection at a volume of 10 ml/kg to produce doses of 20 or 40 mg/kg. CEP-1347/KT-7515 was provided by Kyowa-Hakko Kogyo Co., Ltd. (Tokyo). CEP-1347/KT-7515, was solubilized to a concentration of 6 mg/ml in 30% Solutol (polyethylene glycol hydroxystearate; BASP Corp., Parsippany, NJ) in 70% PBS and stored in 1-ml aliquots at −20°C for up to 4 months. Before an experiment, CEP-1347/KT-7515 was diluted to the appropriate concentration (0.003—0.3 mg/ml) in PBS to achieve a final 5% Solutol concentration. Mice were administered 10 ml/kg of drug solution. CEP-1347/KT-7515 or vehicle was injected s.c. 4 to 6 h before MPTP administration, and then daily until the end of the experiment (7 days). For the delayed treatment experiment, CEP-1347/KT-7515 was administered beginning 7 days after MPTP administration and then continued daily for 7 days. Four hours after the last injection of CEP-1347/KT-7515, mice were sacrificed by exposure to CO2, decapitated, and the midbrain blocked and postfixed in 4% paraformaldehyde overnight. The striata were dissected free, weighed, and frozen on dry ice and stored at −70°C until assayed.

DA, Dihydroxyphenylacetic Acid (DOPAC), and Homovanillic Acid (HVA) analyses. Dopamine, DOPAC, and HVA analyses were conducted by BAS Analytics (Indianapolis, IN). The neostriatal content of DA and its metabolites (DOPAC) and HVA were measured by high-performance liquid chromatography with electrochemical detection as previously described (Sonsalla et al., 1987). Briefly, neostriata were homogenized in 0.1 M perchloric acid (10 mg wet weight per milliliter). Homogenates were centrifuged at 15,000 g for 15 min. The supernatant was analyzed for catecholamine levels. The mobile phase was made of 960 ml of 0.15 M monochloroacetic acid, pH 3.0, containing 50 mg/l EDTA and 200 mg/l sodium oetyl sulfate, 26 ml of acetonitrile, and 21 ml of tetrahydrofuran.

Monoamine concentrations were calculated by comparison of peak height ratios of the samples with those of standards prepared in 0.1 M perchloric acid. Values are expressed as microgram per gram of tissue wet weight.

**Tyrosine Hydroxylase (TH) Assay.** TH enzyme activity was used as a marker for dopaminergic nerve terminals in the striata. This assay has been previously described (Okunu and Fujisawa, 1983). This assay measures the TH-mediated conversion of [14C]tyrosine to L-dopa and the nonenzymatic decarboxylation of L-dopa with subsequent release of [14CO2]. Briefly, striata (6—10 mg wet weight) were homogenized in 20 volumes of 50 mM Tris-Cl buffer (pH 7.4) containing 1 mM EDTA and 0.2% Triton X-100. Homogenates were diluted 1:12 using the homogenization buffer. A total of 100 µl of this diluted stock was incubated in the presence of a 31.3 µM assay cocktail (made fresh: Mes, 0.41 M; catalase, 4000 U; dithiothreitol, 1.48 mM; ascorbate, 2.98 mM; MePhH4, 1.48 mM; [14C]tyrosine, 0.11 µCi, pH 6.2). Samples were incubated at 37°C in a block for 20 min. After incubation, samples were cooled for 30 min on ice. Sample tubes were placed into a wide mouth vial with 400 µl of methylbenzethonium chloride that was in a separate 0.5-ml tube. Methylbenzethonium chloride is used to capture released [14C]CO2. A total of 82 µl of 17 mM potassium ferricyanide and 10 mM p-chloromercuriphenyl sulfonic acid (in Tris buffer, pH 8.0) was injected through the rubber stopper into the homogenate samples. This was done to initiate CO2 release. The samples were incubated at 52°C in a water bath for 30 min. The reaction was halted with addition of 400 µl 0.25 N H2SO4 that was also injected through the rubber stopper in to the sample mixture. The methylbenzenthionum chloride was then counted for radioactivity and captured [14C]CO2 was used as an index of TH enzymatic activity. Protein levels were determined using a micro BCA kit (Pierce Labs, Rockford, IL). Values were normalized to protein levels and are expressed as percentage of control.

**GBR-12935 Binding.** GBR-12935 is a specific ligand for the DA reuptake complex, and binding of the tritiated form to striatal membranes was used as an additional marker for dopaminergic nerve endings. Binding was performed as described by Anderson (1989) with some modifications. Striata were weighed and homogenized in a tissue sonicator in 100 volumes of 50 mM Tris citrate buffer (pH 7.4) containing 120 mM NaCl, and 4 mM MgCl2. Tissue homogenates (900 µl) were incubated with 5 nM [3H]GBR-12935 (45.7 Ci/mmol; NEN Research Products) for 1 h on ice. Final incubation volumes were 1 ml. Incubations took place in 96-well plates designed to hold up to 1.1 ml/well. Incubation proceeded for 1 h at ASPET Journals on June 20, 2017 jpet.aspetjournals.org Downloaded from
Tyrosine Hydroxylase Immunohistochemistry. Fixed frozen mid-brains were sectioned coronally at 30-μm thickness on a cryostat with freezing stage and mounted and dried on slides. Sections were incubated with affinity-purified polyclonal anti-TH antibody (Chemicon, Temecula, CA) at a 1:1000 dilution for 24 h. After washing in PBS, sections were incubated with biotinylated anti-rabbit IgG fraction for 1 h at a 1:100 dilution in PBS. A standard avidin-biotin complex method (Vectastain Elite Kit; Vector Labs, Burlingame, CA) was used and sections were incubated for an additional 30 min with an avidin-biotinylated horseradish peroxidase complex followed by subsequent incubation with diaminobenzidine (1 mg/ml) and 0.03% H2O2.

TH immunoreactive neurons in the substantia nigra pars compacta were counted microscopically as previously described (Jackson-Lewis et al., 1995). Every third 30-μm section (eight sections total) throughout the substantia nigra were assessed for TH immunoreactive neurons for each animal. Cell bodies labeled with anti-TH were counted as neurons if they possessed at least two but no more than six processes. Data from this experiment were expressed as the total number of TH immunoreactive neurons throughout the eight representative sections.

Monoamine Oxidase-B Assay. The effects of CEP-1347/KT-7515 on MAO activities were measured from striata homogenates using a modification of the assay described by White and Stine (1984). Rat striata were homogenized in 0.05 M KH2PO4:K2HPO4 buffer (pH 7.4) at a concentration of 5 mg/ml, based on tissue wet weight. A total of 450 μl of homogenate was preincubated at 37°C in a shaking water bath for 10 min. Forty μl of substrate mixture ([14C]benzylamine; 200 μM) was then added. Samples were incubated for an additional 30 min and the reaction halted with addition of 300 μl of 2 N HCl. One-milliliter ethyl acetate/toluene (1:1) mixture was added to extract products. Samples were centrifuged at 5000 g for 5 min. Tubes were covered and placed on dry ice for 5 min to freeze the aqueous bottom phase. The unfrozen organic layer was counted for radioactivity. Data from this experiment are expressed as nanomoles product formed per milligrams tissue per 30 min.

DA Uptake. Rat striatal synaptsomes were prepared as previously described (Saporito et al., 1992). Rat striata were removed from rat brains and immediately homogenized in 0.3 M sucrose (in water) at a concentration of 10 mg wet weight/ml. This preparation was then centrifuged at 5000g for 7 min. The supernatant was decanted into separate centrifuge tubes. The supernatant was spun at 12000g for 30 min. The pellet was then resuspended in Krebs Ringer-phosphate buffer (in mM: NaCl, 118; NaH2PO4, 15.8; KCl, 4.7; CaCl2, 1.8; MgCl2, 1.2; glucose, 5.6; EDTA, 1.3; ascorbic acid, 1.7; pargyline 0.08; bubbled continuously with 95:5% O2:CO2 at pH 7.4) at a tissue weight. A total of 450 μl of homogenate was preincubated at 37°C in a shaking water bath for 10 min. The tissue was then bubbled continuously for 10 min at 37°C degrees. After preincubation, 10 μl of serially diluted inhibitor was added to each sample. Samples were centrifuged in a table top centrifuge at 10000g for 5 min. Tubes were covered and placed on dry ice for 5 min to freeze the aqueous bottom phase. The unfrozen organic layer was counted for radioactivity. Data from this experiment are expressed as nanomoles product formed per milligrams tissue per 30 min.

Results

CEP-1347/KT-7515 Attenuates MPTP-Mediated Loss of Striatal Terminal Markers. A single subcutaneous injection of MPTP administered at doses of 20 or 40 mg/kg produced deficits of 50 and 80%, respectively, in striatal TH enzyme activity (Figs. 1 and 2). In addition, MPTP (20 mg/kg) produced equivalent losses of three striatal dopaminergic markers. These markers were TH enzyme activity, GBR-12935 binding, and DA (and metabolite) content (Figs. 1 and 2; Table 1). These losses were time dependent with maximal deficits achieved by 3 days post-MPTP injection (data not shown). In the low dose MPTP model, CEP-1347/KT-7515 administration beginning 4 h before MPTP administration (and continuing daily), significantly (p < .05) attenuated the loss of striatal GBR-12935 binding density, TH activity (Fig. 1, A and B) and dopamine content (Table 1) by between 40 and 60%. The maximally effective dose for attenuation of the loss of striatal TH activity and GBR-12935 binding density was 0.3 mg/kg/day. At higher doses, efficacy of CEP-1347/KT-7515 was reduced. At a dose of 40 mg/kg, MPTP produced a
80% loss of striatal TH activity and administration of CEP-1347/KT-7515 significantly ($p < .05$) reduced this loss to 30% (Fig. 2).

**CEP-1347/KT-7515 Reduces MPTP-Mediated Loss of Tyrosine Hydroxylase Immunoreactive Neurons in the Substantia Nigra.** The effect of CEP-1347/KT-7515 on loss of TH immunoreactive neurons in the substantia nigra was also assessed. A 20-mg/kg dose of MPTP produced a nominal loss in the number of TH immunoreactive neurons in the substantia nigra (data not shown). However, a single 40-mg/kg dose of MPTP reduced the number of substantia nigra TH immunoreactive neurons by 80% (Fig. 3). CEP-1347/KT-7515 (0.3 and 3.0 mg/kg/day) significantly ($p < .05$) attenuated the MPTP-mediated loss of TH immunoreactive neuronal number by approximately 50% (Fig. 3).

**CEP-1347/KT-7515 Does Not Increase Striatal Markers with Delayed Dosing.** We designed a study to determine if CEP-1347/KT-7515 could increase dopaminergic markers in MPTP-lesioned animals. In this study, dosing of CEP-1347/KT-7515 was initiated 1 week after MPTP (20 mg/kg) administration and continued for 7 days. This is a time point and dose producing a maximal loss of striatal dopaminergic markers, in the absence of substantia nigra cell body loss. Under this dosing regimen, CEP-1347/KT-7515 (0.03–3.0 mg/kg/day) did not alter the levels of striatal GBR-12935 binding density or TH activity as compared to MPTP-treated vehicle controls (Fig. 4, A and B). Moreover, administration of CEP-1347/KT-7515 (0.3 mg/kg/day) to unlesioned mice did not affect striatal TH activity or GBR-12935 binding density (data not shown).

**CEP-1347/KT-7515 Is Not a MAO-B Inhibitor.** MAO-B inhibitors, including deprenyl, prevent MPTP toxicity by inhibiting the conversion of MPTP to MPP$^+$ (Heikkila et al., 1984). To examine whether CEP-1347/KT-7515 acts in this way, the activity of MAO-B in striatal extracts was assessed in the presence of CEP-1347/KT-7515 and l-deprenyl and clorgyline, selective inhibitors for MAO-B and -A, respectively. As expected (Fig. 5), l-deprenyl but not clorgyline, inhibited MAO activity with an IC$_{50}$ value of 22 nM (Heikkila et al., 1984b). CEP-1347/KT-7515 had no effect on MAO-B enzyme activity up to a concentration of 10 μM (Fig. 5).

**CEP-1347/KT-7515 Is Not a Dopamine Uptake Inhibitor.** DA uptake inhibitors are effective neuroprotective agents in the MPTP mouse model (Javitch et al., 1985). DA uptake inhibitors prevent MPTP-mediated toxicity by blocking the active uptake of MPP$^+$ into dopaminergic neurons via the DA transporter. CEP-1347/KT-7515 was assessed for its ability to inhibit DA uptake into striatal synaptosomes. As seen in Fig. 6, uptake was inhibited by the selective dopamine uptake blocker GBR-12909 at a concentration (IC$_{50}$ = 8 nM) that matched the reported literature value for this inhibitor (Anderson, 1987). In contrast, incubation of CEP-1347/KT-7515 at concentrations up to 3 μM with the synaptosomal sample did not significantly affect [3H]DA uptake.

**Discussion**

CEP-1347/KT-7515 promotes neuronal survival and is neuroprotective in a variety of primary neuronal culture systems and in models of neurodegeneration in vivo. In the current studies, CEP-1347/KT-7515 attenuated the MPTP-mediated degeneration of nigrostriatal dopaminergic nerve terminals and cell bodies. Neuroprotective activity was observed with peripheral administration of CEP-1347/KT-7515 beginning 4 h before MPTP (and then daily) but was not evident when dosing was initiated after maximal loss of these dopaminergic neurons (7 days after MPTP administration). CEP-1347/KT-7515 did not inhibit MAO-B activity or the dopamine transporter, suggesting that CEP-1347/KT-7515 does not act by interfering with the conversion of MPTP to MPP$^+$ or uptake of MPP$^+$ into dopaminergic neurons.

In mice, MPTP administration produces a well-described nigrostriatal dopaminergic degeneration. In our studies, a 20-mg/kg dose of MPTP produced consistent and equivalent losses of striatal TH activity, GBR-12935 binding density (which reflects the presence of the dopamine transporter) and DA (and metabolite content) in the absence of any loss of TH immunoreactive neurons. Systemic administration of CEP-1347/KT-7515 significantly attenuated the MPTP-mediated decrease in all striatal dopaminergic parameters as compared to MPTP-treated vehicle controls (Table 1; Fig. 1). The preservation of these three independent markers suggests that CEP-1347/KT-7515 administration resulted in dopaminergic terminal preservation, rather than preservation or an increase of a single dopaminergic parameter.

At a higher dose of MPTP (40 mg/kg) there was a near
complete loss of TH activity and a dramatic loss of TH immunoreactive neurons in the substantia nigra. CEP-1347/KT-7515 partially ameliorated the loss of TH activity and GBR-12935 binding in the striatum (Fig. 2) and significantly reduced the loss of TH immunoreactive neuronal cell bodies in the substantia nigra (Fig. 3). These data indicate that CEP-1347/KT-7515 protects both degenerating dopaminergic cell bodies and striatal nerve terminals. The effective dose-range for CEP-1347/KT-7515 was similar for the striatal and substantia nigra measures, suggesting that the neuroprotective activities of CEP-1347/KT-7515 on both dopaminergic parameters are related.

Fig. 4. CEP-1347/KT-7515 does not affect dopaminergic terminal markers when administration is delayed 7 days after MPTP injection. MPTP was administered at a dose of 20 mg/kg (s.c.). CEP-1347/KT-7515 administration was initiated 7 days after MPTP injection and continued daily for 7 additional days. Striatal TH activity (A) and GBR-12935 binding density (B) were assessed 4 h after the last injection of CEP-1347/KT-7515.

Fig. 3. CEP-1347/KT-7515 attenuates the loss of substantia nigra TH immunoreactive neurons after MPTP lesion. MPTP was administered s.c. at a dose of 40 mg/kg. CEP-1347/KT-7515 was administered 4 h before MPTP administration and then every day until the end of the experiment. Mice were sacrificed 7 days post-MPTP administration and 4 h after the last injection of CEP-1347/KT-7515 or vehicle. Brains were postfixed and TH immunoreactivity was assessed as described under Materials and Methods. Eight representative sections were counted throughout the substantia nigra. A, dose-response of CEP-1347/KT-7515; data are combined from two independent experiments. n = eight per treatment group. *Indicates statistical difference (p < .05) from MPTP-treated vehicle controls (Vehicle). Representative microphotographs of substantia nigra: B, control; C, MPTP (40 mg/kg); D, CEP-1347/KT-7515 treated (0.3 mg/kg/day).
Results to date indicate that CEP-1347/KT-7515 protects neurons from neurodegeneration and does not increase the expression of neuronal phenotypic markers. Previously we have shown that CEP-1347/KT-7515 prevented the ibotenic acid lesion induced loss of cholinergic neurons and Fluoro-Gold labeled cortically projecting neurons of the nbm when dosing was initiated near the time of lesion but not after complete (4 day postlesion) neuronal degeneration (Saporto et al., 1998). In the current studies, CEP-1347/KT-7515 was effective if administration began 4 h before MPTP administration but was ineffective if administration was delayed until 7 days after MPTP administration, i.e., a time point in which there was complete dopaminergic terminal degeneration. These data are consistent with the hypothesis that CEP-1347/KT-7515 prevents injury-induced neurodegeneration and does not increase the expression of neuronal phenotypic markers. The effective dose-range for neuroprotection of CEP-1347/KT-7515 in the MPTP model is essentially identical with the effective dose-range discovered in our previous in vivo studies (0.1–1.0 mg/kg/day), suggesting that the neuroprotective mechanisms are similar for both lesions.

Several compounds prevent the loss of injured nigrostriatal dopaminergic neurons in vivo. However, these compounds appear to act very differently from CEP-1347/KT-7515. For example, the protein growth factor, glia-derived neurotrophic factor (GDNF), increases dopaminergic functional parameters and promotes neuronal survival in MPTP-treated mice and rats subjected to 6-hydroxydopamine lesion or medial forebrain bundle transection (Beck et al., 1995, Sauer et al., 1995, Tomac et al., 1995). In contrast to CEP-1347/KT-7515, GDNF increases dopaminergic functional parameters when administered after loss of dopaminergic neurons has occurred (Tomac et al., 1995). The neuroimmunophilin GPI 1046, promotes dopaminergic neuronal sprouting in MPTP-treated mice and 6-hydroxydopamine lesioned rats, and as with GDNF, increases striatal TH activity after complete dopaminergic terminal degeneration (Steiner et al., 1997).

However, its effect on dopaminergic neuronal survival in the substantia nigra has not been described. DA uptake blockers and MAO-B inhibitors prevent formation of MPP\(^+\) and uptake of MPP\(^+\) into dopaminergic neurons, respectively and, in turn, inhibit MPTP-mediated nigrostriatal dopaminergic degeneration (Heikkila et al., 1984; Javitch et al., 1985, Sonnalla et al., 1987). CEP-1347/KT-7515 up to a concentration of 3 \(\mu M\), did not inhibit MAO-B activity or the dopamine transporter, suggesting that the neuroprotective activity of CEP-1347/KT-7515 in the MPTP mouse model does not involve inhibition of the conversion of MPTP to MPP\(^+\) or affect accumulation of MPP\(^+\) into dopaminergic neurons. Interestingly, \(L\)-deprenyl prevents neuronal degeneration in various in vivo neurodegenerative models by a mechanism that appears to be independent of its ability to inhibit MAO-B (Mytilineou and Cohen 1985; Ansari et al., 1993; Tatton and Greenwood, 1991). In a mouse MPTP model, \(L\)-deprenyl attenuates the MPTP-mediated loss of TH immunoreactive neurons in the substantia nigra in a manner that appears to be similar to that of CEP-1347/KT-7515 (Tatton et al., 1991).

The biochemical sequelae that lead to neuronal death after MPP\(^+\) inhibition of mitochondrial respiration are not known. However, there is evidence that MPP\(^+\) produces an apoptotic response in PC12 cells, primary cultures of mesencephalon and in vivo (Hartley et al., 1994; Mochizuki et al., 1994, Tatton and Kish, 1997). Interestingly, we have evidence that CEP-1347/KT-7515 inhibits apoptotic death in various neuronal systems. For example, CEP-1347/KT-7515 promotes the survival of motor neurons in developing chick embryo and inhibits programmed cell death of motor neurons of the spinal nucleus bulbocaverous in female postnatal rats (Glicksman et al., 1998). These data suggest that the neuroprotective activity of CEP-1347/KT-7515 (including that seen in the MPTP mouse model) may be related to its ability to interfere with an event that leads to apoptosis.

Recently, we have defined a specific apoptotic signaling pathway that CEP-1347/KT-7515 interacts with. In primary cultures of motor neurons, CEP-1347/KT-7515 inhibits the activation of the Jnk signaling pathway and promotes motor neuronal survival at similar low nanomolar concentrations (Maroney et al., 1998). There is substantial evidence that activated (phosphorylated) Jnk, and the Jnk substrate, c-jun, mediate some forms of neuronal apoptosis. For example, in differentiated PC12 cells, dominant-negative Jnk as well as
overexpression of an endogenous inhibitor of Jnk (termed Jnk inhibitor protein) inhibits apoptosis associated with NGF withdrawal (Ham et al., 1995, Xia et al., 1995, Dickens et al., 1997). Microinjection of a c-jun neutralizing antibody to sympathetic neurons prevents NGF withdrawal-induced apoptosis in cultured sympathetic ganglia (Estus et al., 1994). In vivo, mice deficient in Jnk3 (a Jnk isoform) are resistant to kainic acid-induced seizures and apoptosis in the hippocampus (Yang et al., 1997). These data indicate that Jnk activation is a necessary component of some forms of neuronal apoptotic death.

The Jnk signaling pathway may be involved in injury-induced nigrostriatal dopaminergic degeneration. For example, c-jun levels in the substantia nigra are increased after 6-hydroxydopamine induced-injury to striatal dopaminergic afferents (Jenkins et al., 1993). Recently, it has been demonstrated that levels of phosphorylated c-jun (as well as c-jun) are increased after nigrostriatal transaction at a time point before neuronal loss (Brecht et al., 1994; Herdegen et al., 1998). Importantly, MPTP administration to mice has been found to increase c-jun immunoreactivity in degenerating dopaminergic neurons of the substantia nigra (Nishi et al., 1997). These data, together with the known ability of CEP-1347/KT-7515 to inhibit Jnk activation, suggest that Jnk activation may be a contributing factor in MPTP-mediated dopaminergic degeneration in mice.

The MPTP mouse model is one of the more widely used animal models of nigrostriatal degeneration and mimics many of the neurodegenerative aspects of Parkinson’s disease. In our studies, CEP-1347/KT-7515 attenuated MPTP-mediated nigrostriatal dopaminergic degeneration in mice. These data, together with the known ability of CEP-1347/KT-7515 to prevent apoptosis and to inhibit Jnk activation, may be additional evidence that MPTP elicits apoptosis in nigrostriatal dopaminergic neurons and may implicate activation of the Jnk pathway in MPTP-mediated dopaminergic degeneration. Moreover, these results may suggest that CEP-1347/KT-7515 may have potential in Parkinson’s disease by slowing or preventing the progression of the disease.

References

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