Systemic Infusion of Naloxone Reduces Degeneration of Rat Substantia Nigral Dopaminergic Neurons Induced by Intranigral Injection of Lipopolysaccharide

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ABSTRACT

A massive degeneration of dopamine-containing neurons in the substantia nigra (SN) in the midbrain is characteristic of Parkinson’s disease. Inflammation in the brain has long been speculated to play a role in the pathogenesis of this neurological disorder. Recently, we reported that treatment of primary rat mesencephalic mixed neuron-glia cultures with lipopolysaccharide (LPS) led to the activation of microglia, resident immune cells of the brain, and subsequent death of dopaminergic neurons. The LPS-induced degeneration of dopaminergic neurons was significantly attenuated by the opiate receptor antagonist (−)-naloxone and its inactive isomer (+)-naloxone, with equal potency, through an inhibition of microglial activation and their production of neurotoxic factors. In this study, injection of LPS into the rat SN led to the activation of microglia and degeneration of dopaminergic neurons: microglial activation was observed as early as 6 h and loss of dopaminergic neurons was detected 3 days after the LPS injection. Furthermore, the LPS-induced loss of dopaminergic neurons in the SN was time- and LPS concentration-dependent. Systemic infusion of either (−)-naloxone or (+)-naloxone inhibited the LPS-induced activation of microglia and significantly reduced the LPS-induced loss of dopaminergic neurons in the SN. These in vivo results combined with our cell culture observations confirmed that naloxone protects dopaminergic neurons against inflammation-mediated degeneration through inhibition of microglial activation and suggest that naloxone would have therapeutic efficacy in the treatment of inflammation-related neurological disorders. In addition, the inflammation-mediated degeneration of dopaminergic neurons in the rat SN resulting from the targeted injection of LPS may serve as a useful model to gain further insights into the pathogenesis of Parkinson’s disease.

Parkinson’s disease is one of the major neurodegenerative disorders that affect millions of people each year. The characteristic of this disease is the gradual degeneration of the dopaminergic neurons in the substantia nigra (SN) pars compacta of the midbrain (Olanow and Tatton, 1999). Although a tremendous effort has been devoted to studying this neurological disorder in the past decades, the mechanism(s) underlying the progressive loss of a highly selective group of neurons (i.e., dopamine-containing neurons) in a very specific region of brain (i.e., SN) is not yet fully understood. The discovery that the active metabolite of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), 1-methyl-4-phenylpyridinium, killed dopaminergic neurons both in cell culture systems and experimental animal models has significantly advanced our understanding of Parkinson’s disease (Langston et al., 1984). Nevertheless, the knowledge gained from these studies cannot adequately explain the progressive and highly specific loss of a subgroup of dopaminergic neurons during the development of this disease.

In recent years, increasing evidence has strongly suggested a role for inflammation in the brain in the pathogenesis of a variety of neurodegenerative diseases, including Parkinson’s and Alzheimer’s diseases, amyotrophic lateral sclerosis, and the AIDS dementia complex (McGeer et al., 1988; Rogers et al., 1988; Raine, 1994; Glass and Johnson, 1996). Inflammatory responses in the brain are now thought to be mainly associated with activity of glial cells. In particular, microglia, a subset of the glial cells, are considered to be the resident immune cells in the brain and are the most responsive to immunological challenges. Activated microglia produce a

ABBREVIATIONS: SN, substantia nigra; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NO, nitric oxide; LPS, lipopolysaccharide; TH, tyrosine hydroxylase; OX-42, anti-CR3 complement receptor antibody; Neu-N, neuron-specific nuclear protein; VTA, ventral tegmental area; TNFα, tumor necrosis factor-α; IL-β, interleukin-1β; 6-OH DA, 6-hydroxyl dopamine.
host of proinflammatory and cytotoxic factors, including cytokines, nitric oxide (NO), reactive oxygen species, and arachidonic acid metabolites (Chao et al., 1992; Dickson et al., 1993; Lee et al., 1993; Brosnan et al., 1994; Minghetti and Levi, 1995). Although the combination of these factors is thought to contribute to the neurodegenerative processes both in cell culture systems and animal models, the precise mechanisms of action remain to be elucidated.

Recently, using mixed neuron-glia cultures of the embryonic rat mesencephalon, we reported that treatment with lipopolysaccharide (LPS) stimulated microglia to release proinflammatory and cytotoxic factors that led to the eventual loss of dopaminergic neurons in the cultures (Liu et al., 2000). Naloxone, an antagonist of opioid receptors, protected dopaminergic neurons against inflammation-mediated damage through inhibition of microglial activation. We have extended our in vitro experiments to animal studies and report herein that intranigral injections of LPS into rat SN result in a rapid activation of microglia followed by the loss of tyrosine hydroxylase (TH)-immunoreactive neurons. Systemic infusion of the opioid receptor antagonist (-)-naloxone or its inactive stereoisomer (+)-naloxone inhibited microglial activation and protected dopaminergic neurons against LPS-induced damage.

Experimental Procedures

Animals. Male Fischer 344 rats (225–250 g) were obtained from Charles River Laboratories (Raleigh, NC) and kept on a 12-h light/dark cycle with ad libitum access to food and water. Rats were acclimated to their environment for 2 weeks before the experiments.

Materials. (-)-Naloxone was purchased from Research Biochemicals International (Natick, MA). The enantiomer (+)-naloxone was a generous gift from National Institute of Drug Abuse (Rockville, MD). Monoclonal antibodies against the CR3 complement receptor (OX-42) and the neuron-specific nuclear protein (Neu-N) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA) and Chemicon (Temecula, CA), respectively. The polyclonal anti-TH antibody was a gift from Dr. John Reinhard of Glaxo-Wellcome (Research Triangle Park, NC). LPS (Escherichia coli 0111:B4) and endotoxin-tested and sterile PBS were purchased from Sigma (St. Louis, MO). Alzet mini-osmotic pumps were purchased from Alza Corp. (Palo Alto, CA). Sodium pentobarbital was obtained from Abbott Laboratories (North Chicago, IL). VECTASTAIN ABC reagents, NOVA Red color developer, and biotinylated secondary antibodies were purchased from Vector Laboratories (Burlingame, CA).

LPS Injection. Rats were anesthetized with sodium pentobarbital (50 mg/kg) and positioned in a small-animal stereotaxic apparatus. For injection of LPS into the SN pars compacta, the following coordinates were used: 4.8 mm posterior to bregma, 1.7 mm lateral to the midline, and 8.2 mm ventral to the surface of the skull (Paxinos and Watson, 1986). LPS was prepared as a stock solution of 5 µg/ml in sterile PBS and stored in small aliquots at 4°C. Each rat received an injection of LPS dissolved in 2 µl of PBS into one side of the brain and 2 µl of PBS into the opposite side. The injection was conducted over a period of 2 min and controlled by a motorized microinjection pump. After the injection, the needle was kept in place for 2 min.

Systemic Infusion of Naloxone. Freshly prepared solutions of naloxone were used for each experiment. Solutions of either naloxone isomer were prepared in PBS, sterile filtered through a 0.2-µm syringe filter, and carefully loaded into 2-ml Alzet mini-osmotic pumps. Afterward, the minipumps were implanted s.c. on the dorsal side of the neck of anesthetized rats. Implantation of minipumps was done 16 to 24 h before the injection of LPS. Control animals were implanted with minipumps loaded with vehicle PBS alone.

Immunohistochemistry. Rats were perfused transcardially with PBS followed by ice-cold 4% paraformaldehyde in PBS (pH 7.4). Brains were removed, postfixed for 2 days at 4°C in 4% paraformaldehyde in PBS, and cryoprotected for 2 days at 4°C in 30% sucrose/1% paraformaldehyde. Coronal sections (35 µm) were cut through the nigral complex using a microtome and stored in PBS containing 0.1% sodium azide. Immunohistochemical staining was performed as previously described (Perez-Otano et al., 1996). Dopaminergic neurons were recognized with an anti-TH polyclonal antibody, neuronal cell bodies with an antibody against Neu-N, and microglia with a monoclonal antibody against OX-42. Briefly, floating brain sections were sequentially incubated with the following reagents: 1% hydrogen peroxide (10 min), blocking solution (PBS containing 1% BSA, 0.4% Triton X-100, and 4% appropriate serum; 40 min), primary antibody diluted in blocking solution (anti-Neu-N, 1:1,000; anti-TH, 1:20,000; or OX-42 antibody, 5 µg/ml, overnight, 4°C), biotinylated secondary antibodies (1:227 in PBS containing 0.3% Triton X-100; 2 h), and VECTASTAIN ABC reagents (40 min). Sections were washed two or three times in between steps. The bound antibody complex was visualized with 3,3′-diaminobenzidine (Sigma). For double labeling, sections were stained with the anti-Neu-N antibody (using NOVA Red as developer, red color) and then with the anti-TH antibody (using 3,3′-diaminobenzidine as developer, brown color). The images were analyzed with a Zeiss microscope connected to a charge-coupled device camera (DAGE-MTI, Michigan City, IN) and analyzed with the MetaMorph Image System (Universal Image and Co., West Chester, PA).

Visual Quantification of Nigral TH-Positive Neurons. For all studies, every effort was taken to ensure the uniformity of the brain sections used. Wherever uneven sectioning was discovered, the entire brain was excluded. To determine the extent of damage, brain sections were taken through the entire nigral complex (Fig. 2). Every one of the approximately 50 sections collected was used to visually count the number of nigral TH-positive neurons under a microscope. In subsequent experiments, sections 12 through 35 (rostral to cor- dial: 4.52 to 5.36 mm posterior to bregma) of the 50 sections were selected. In some cases, all 24 of the selected sections were used for quantification of nigral TH-positive neurons. In other cases, every other of the 24 sections was used for staining for TH-positive neurons and the in-between sections for staining for Neu-N-positive neurons or for microglial activation using the OX-42 antibody. Quantification of the nigral TH-positive neurons was performed by visually counting the number of nigral TH-positive neuronal cell bodies under a microscope by three individuals in a blind fashion. Unless otherwise stated, the number of TH-positive neurons in the nigral region of the side of brain injected with LPS was first calculated as a percentage of those on the opposite side injected with PBS for each section, and then an average of the multiple sections (12–24) for each brain was deduced.

Statistical Analysis. The data are expressed as mean ± S.E. Statistical significance was assessed by an ANOVA followed by Bonferroni’s t test using the StatView program (Abacus Concepts, Inc., Berkeley, CA). A value of P < .05 was considered statistically significant.

Results

LPS-Induced Neurodegeneration in Rat SN. LPS (2.5 µg in 2 µl of PBS) was stereotaxically injected into the rat SN to examine its effect on neurodegeneration. Five days later, the brain was removed, and coronal sections were taken through the nigral complex. The sections were double immunostained with an antibody against a neuronal nuclear protein (Neu-N) to detect neurons in general and an antibody against TH to specifically detect dopaminergic neurons. As shown in Fig. 1, immunostaining of a coronal section of the rat brain with the anti-Neu-N antibody revealed neuronal...
cell body staining throughout the entire brain. However, the dopaminergic neurons in the SN and the ventral tegmental area (VTA) were detected by both the anti-Neu-N antibody (cell body) and the anti-TH antibody (both cell body and dendrites). After LPS injection into the SN, a significant loss of both the Neu-N-positive and TH-positive neurons in that region was observed compared with the same region on the opposite side of the brain injected with PBS (Fig. 1A). Besides a marked loss of neuronal cell bodies, the extensive fiber network of the TH-positive neurons in the SN was nearly completely destroyed (Fig. 1B). However, the number and integrity of the TH-positive neurons in the VTA adjacent to the SN were not significantly affected by LPS injection (Fig. 1, A and B). To determine the extent and pattern of loss of dopaminergic neurons throughout the SN, a series of coronal sections were taken through the entire nigral complex (rostro to caudal), stained with the anti-TH antibody, and TH-positive neurons in the SN of each section were counted. As shown in Fig. 2, an across-the-board loss of TH-positive neurons was observed, exhibiting a pattern very similar to that observed in patients of Parkinson’s disease (Damier et al., 1999). In subsequent experiments, of the approximately 50 sections taken from the entire nigral complex of each brain, usually only the sections 12 to 35 (rostro to caudal) were selected to count the number of TH-immunoreactive neurons.

**LPS-Induced Neurodegeneration in Rat SN Was Concentration- and Time-Dependent.** The concentration dependence of LPS-induced loss of TH-positive neurons in the SN was examined by injecting various amounts of LPS (0.1–5 μg) into the rat SN. Five days after injection of 0.5 μg of LPS, a 20% loss of TH-positive neurons in the SN was observed (Fig. 3A). Injection of larger amounts of LPS resulted in a greater loss of TH-positive neurons and 5 μg of LPS destroyed 95% of the TH-positive neurons in the area (Fig. 3A). The time dependence of the LPS-induced neurodegeneration in the SN was examined by injecting 2.5 μg of LPS into the SN, and rats were sacrificed at different time points. As shown in Fig. 3B, no apparent loss of TH-positive neurons in the SN was observed at 6, 12, or 24 h postinjection of 2.5 μg of LPS. However, 3 days after an injection of 2.5 μg of LPS, a loss of 45% of TH-positive neurons in the SN was detected (Fig. 3B). The loss reached a maximum (85%) 5 days after the injection of LPS and was maintained at that level for up to 28 days after the LPS injection (Fig. 3B). Staining of adjacent brain sections from rats sacrificed 28 days after LPS injection with either the anti-TH antibody or anti-Neu-N antibody confirmed that the disappearance of TH-like immunoreactivity in the SN was, in fact, due to a comparable loss of neuronal cell bodies (Fig. 4).

**Time Dependence of LPS Injection-Induced Activation of Microglial Cells in SN.** Activation of microglia is frequently observed during the pathogenesis of neurodegenerative diseases and has been thought to play an important role in the progression of neurodegeneration. To study microglial activation, brains were sectioned through the SN complex and immunostained with the antibody OX-42 raised against the CR3 complement receptor, a specific marker of microglial activation (Kreutzberg, 1996). Activation of microglia revealed by immunostaining with the OX-42 antibody is characterized by an increase in both the size of the cells and the intensity of the staining. Morphologically, resting stage microglia in vivo exhibit the “ramified” shape. Activation of microglia drastically alter their morphological appearance...
number of animals used for each group. Results were expressed as a percentage of the number of TH-immunoreactive neurons in the SN. Every other of the 24 sections was immunostained with an anti-TH antibody, and the number of TH-immunoreactive neurons in the SN was counted. Results were expressed as a percentage of the number of TH-positive neurons of the side of brain injected with saline as described under Experimental Procedures. Numbers in parentheses indicate the number of animals used for each group. *P < .01 and **P < .001 compared with the corresponding control group.

Infusion of Naloxone Inhibited LPS-Induced Microglial Activation and Reduced LPS-Induced Neurondegeneration. In rat midbrain neuron-glia cultures, pretreatment of cultures with naloxone before treatment of LPS significantly inhibited LPS-induced microglial activation and offered significant protection to dopaminergic neurons against LPS-induced degeneration (Liu et al., 2000). To examine the effect of naloxone on LPS-induced activation of microglia and degeneration of dopamine-containing neurons in rat SN, (–)-naloxone (1 mg/day) was systemically infused into the rat 24 h before the injection of LPS (2.5 μg). Twenty-four hours after the injection of LPS, brain sections were taken through the nigral complex and immunostained with the OX-42 antibody. As shown in Fig. 6, the majority of the OX-42-immunoreactive microglial cells in the brains of rats infused with (–)-naloxone before LPS injection exhibited a morphology very much resembling that of the ramified microglia with only a small portion had the shapes of intermediate rod-like and partially activated microglia. As demonstrated in Fig. 6, similar results were observed in rats infused with (+)-naloxone (1 mg/day) before LPS injection (2.5 μg; 24 h). These results indicated that naloxone isomers significantly reduced the LPS-induced activation of microglia, consistent with our observation in the rat midbrain neuron-glia cultures (Liu et al., 2000).

To examine the effect of naloxone on the LPS-induced degeneration of TH-positive neurons in the SN, systematic infusion of naloxone (0.11–1.0 mg/day) was initiated 16 to 24 h before the injection of LPS. Five days after the injection of 2.5 μg of LPS and concomitant infusion of naloxone for the same 5-day period, the brains were removed, sectioned, and immunostained for TH-positive neurons. As shown in Fig. 5, infusion of (–)-naloxone resulted in a dose-dependent protection of TH-positive neurons against LPS-induced degeneration. In rat infused with 0.33 and 1 mg/day (–)-naloxone, 40 and 70%, respectively, of the TH-positive neurons in the SN remained accounted for compared with only 15% in rats injected with 2.5 μg of LPS without the infusion of naloxone but with vehicle PBS alone (Fig. 7A). Furthermore, a comparable portion (70%) of TH-positive neurons in the SN remained accounted for between rats infused with 1 mg/day (–)-naloxone and those with same quantity of its stereoisomer, (+)-naloxone, which is unable to bind opiate receptors (Fig. 7A). Morphologically, infusion of either (–)-naloxone or (+)-naloxone (1 mg/day) significantly reduced the degeneration of TH-positive neuronal fibers in the SN induced by LPS (2.5 μg; 5 days; Fig. 7B). Infusion of either the (–)-naloxone or (+)-naloxone alone (1 mg/day; 6 days), or vehicle PBS did not show any effect on the morphology and numbers of
TH-positive neurons in the SN compared with nontreated rats (n = 3; data not shown).

Discussion

In this study, we have shown that injection of sub-to-low microgram quantities (0.5–5 μg) of LPS into the SN pars compacta of adult rats resulted in a concentration- and time-dependent degeneration of dopaminergic neurons in that region. LPS induced significant activation of microglia, the resident immune cells in the brain, and microglial activation preceded the apparent neuronal degeneration. Naloxone, originally considered solely as an antagonist of the classical opioid receptors, significantly reduced the LPS-induced activation of microglia and the subsequent neurodegeneration. The neuroprotective effect of naloxone might not be directly related to the activities of classic opioid receptors because both the opioid receptor antagonist (−)-naloxone and its ineffective stereoisomer (+)-naloxone were equally potent. The results observed in the animal studies herein are an important extension and confirmation of our in vitro studies with primary rat mesencephalic neuron-glia cultures (Liu et al., 2000).

Among the various subpopulations of dopaminergic neurons in the brain, the highly selective and gradual loss of the dopaminergic neurons in the SN pars compacta is characteristic of the development of Parkinson’s disease (Olanow and Tatton, 1999). Despite decades of research, the mechanism underlying this unique pathological phenomenon is not fully understood. The discovery of direct neurotoxins such as MPTP has shed significant light on the research without providing a fully satisfactory explanation for the highly selective and progressive loss of dopamine neurons (Langston et al., 1984). Recently, increasing evidence has suggested a
role for inflammation in the pathogenesis of this disease. Although no causal relationship has been established, epidemiological studies suggest that post-traumatic brain injury related-inflammation may contribute to the development of this neurological disorder (Nayernouri 1985; Factor et al., 1988; Jellinger, 1989; Stern, 1991), probably best exemplified by case studies of certain ex-boxers with Parkinsonian syndromes (Friedman, 1989; Davie et al., 1995). Because microglia are the principal immune cells in the brain and they readily become activated in response to injury, infection, or inflammation (Streit et al., 1988; Kreutzberg, 1996), it is highly conceivable that activation of microglia plays a key role in the pathogenesis of Parkinson's diseases. In fact, activation of microglia has been observed in Parkinson’s disease as well as other degenerative neurological disorders (McGeer and McGeer, 1995; Matyszak, 1998). Furthermore, the presence and especially the activity of microglia may help explain the highly selective degeneration of dopamine neurons in the SN during the development of Parkinson’s disease. First, the abundance of microglia in the SN appeared to be significantly higher than that in other brain regions (Lawson et al., 1990; Kim et al., 2000). Second, both in vitro and in vivo studies indicated that compared with counterparts in other brain regions, neurons in the SN were most sensitive to inflammation-mediated damage (Kim et al., 2000). The sensitivity of neurons was directly related to the quantity of microglia present in the in vitro cultures because insensitive cultures with few microglia would become sensitive if reconstituted with additional microglia (Kim et al., 2000). Third, dopamine neurons in the SN appeared to be more vulnerable to injury than those in other brain areas perhaps due to a reduced antioxidant capacity (Jenner and Olanow, 1996), a potentially key regulator in cellular sensitivity to assault (Liu et al., 1998). Finally, a recent report further suggested the involvement of the activity of microglia and astroglia in the neurotoxin MPTP-elicited neuronal damage in the SN (Kurkowska-Jastrzebska et al., 1999). The unique feature in microglia abundance and dopamine neurons with increased sensitivity in the SN, in combination with insults from environmental toxins, infections, and genetic predisposition may all be part of the complex etiology of Parkinson’s disease (Calne et al., 1984; Stern et al., 1991; Hirsch et al., 1998).

At the cellular level, microglial activation involves a dramatic morphological change from a ramified resting form to a fully activated ameboid appearance with a significant increase in the expression of major histocompatibility complex molecules and complement type 3 receptor (Streit et al., 1988; Kreutzberg, 1996). In fact, the morphology of microglia as revealed by immunostaining with the OX-42 antibody, which recognizes the complement type 3 receptor, has served as a very useful and fairly reliable indicator of their activation status (Kreutzberg, 1996). In addition to the resting ramified and fully activated ameboid morphology, partially activated microglia exhibited a rod-like shape (Kreutzberg, 1996). Indeed, this study again demonstrated the time-dependent and activation stage-related morphological changes of microglia in response to immunological stimuli (Fig. 5).

At the molecular level, activated microglia produce a variety of proinflammatory and cytotoxic factors, including the cytokines tumor necrosis factor-α (TNFα), interleukin-1β (IL-1β), the free radical NO, reactive oxygen species, and eicosanoids, metabolites of arachidonic acid (Chao et al., 1992; Dickson et al., 1993; Lee et al., 1993; Brosnan et al., 1994; Minghetti and Levi, 1995; Liu et al., 2000). These factors released by activated microglia will impact on neurons, causing their eventual degeneration through mechanism not completely understood. Using rat mesencephalic neuron-glial cultures, we have recently demonstrated that treatment of the cultures with the bacterial endotoxin LPS induced the activation of microglia that in turn produced large quantities of TNFα, IL-1β, NO, and superoxide free radicals (Liu et al., 2000). The LPS-induced production of these proinflammatory and cytotoxic factors was followed first by a loss of function of the dopaminergic neurons (dopamine uptake ability) and then by structural damage to the dopaminergic neurons (loss of dendrites and neuronal perikarya). Inhibition of LPS-induced microglial activation by agents such as naltrexone resulted in a much reduced production of TNFα, IL-1β, NO, and especially superoxide free radical and offered significant protection of dopaminergic neurons against LPS-induced.
damage (Liu et al., 2000). In analogy to the cell culture observations, in this study, injection of LPS to the SN of rat brain resulted in a rapid activation of microglia at 6 h after the LPS injection. However, the loss of TH-positive neurons was only observed 3 days post-LPS injection. This temporal relationship between microglial activation and neurodegeneration strongly suggests that microglial activation precedes the degeneration of dopaminergic neurons in the SN. This notion is further supported by the fact that infusion of naloxone inhibited LPS-induced microglial activation and subsequent neurodegeneration. It will be of tremendous interest to sort out the factors originated from activated microglia that contribute to degeneration of dopaminergic neurons in this animal model. Equally important will be the understanding of the detailed mechanism of action responsible for the microglial activation-inhibitory and neuroprotective effect of naloxone in this system.

Degeneration of dopaminergic neurons induced by targeted injection of LPS may serve as a useful animal model, in relation to brain inflammation, to help us further understand the pathogenesis of Parkinson’s disease (Castano et al., 1998; this study). Previously, other agents such as 6-hydroxy dopamine (6-OH DA) have been used to induce degeneration of dopamine neurons in SN. When compared at an equal molar basis, LPS might be more potent than 6-OH DA in inducing the death of dopamine neurons. In this study, an injection of 2.5 μg of LPS (average mol. wt. 3000–4000) resulted in a loss of 80% of the dopamine neurons, whereas 10 μg of 6-OH DA (mol. wt. 169) was required to achieve significant lesion (Li et al., 1990). It is also worth noting that, in this study, although the injection of LPS into the SN caused significant damage to dopaminergic neurons in that area, the dopaminergic neuron in VTA, an area adjacent to the SN, did not appear to be affected. It is not clear whether this suggests any selectivity in LPS-induced toxicity between dopaminergic neurons in the SN and those in the VTA. Additional studies are certainly warranted. Furthermore, LPS injection into the SN resulted in a general loss of neurons in that region; it remains to be determined whether lower doses of LPS injected and/or long-term infusion of LPS will give rise to any preferential degeneration of dopaminergic neurons over other types of neurons in the SN.

Naloxone was initially synthesized as a potent yet nonselective antagonist of the classical opioid receptors (types μ, δ, and κ), and its opioid receptor antagonistic property is stereospecific: only (−)-naloxone is effective and the (+)-enantio- mer is considered inert (Iijima et al., 1978). Administration of (−)-naloxone has been found to have beneficial effects in animal models of stroke, myocardial and brain ischemia, and traumatic injuries of the brain and spinal cord (Hosobuchi et al., 1982; Fallis et al., 1983; Faden and Salzman, 1992; Kan et al., 1992). However, subsequent studies have found (+)-naloxone to be effective in certain cases. For example, both naloxone isomers effectively reduced cocaine-induced hyperactivity in mice and protected murine cortical neurons from N-methyl-D-aspartate-mediated neurotoxicity (Kim et al., 1987; Chatterjee et al., 1996). In both in vitro (Liu et al., 2000) and in vivo studies described herein, the naloxone stereoisomers were equally effective in protecting neurons against inflammation-mediated damage. Their neuroprotective effects are unlikely linked directly to the classic opioid receptor systems and instead most likely due to their ability to inhibit the activation of brain immune cells (i.e., microglia) and their production of proinflammatory and cytotoxic factors. These inflammation-related neuroprotective effects of naloxone can be further exploited to design agents with therapeutic potential for the treatment of neurodegenerative disorders.