Intrastriatal implantation of interleukin-1

Reduction of parkinsonism in rats by enhancing neuronal sprouting from residual dopaminergic neurons in the ventral tegmental area of the midbrain

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Intrastriatal implantation with dopaminergic or nondopaminergic tissue can elicit behavioral recovery in parkinsonian animals. Because in these animals, especially in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned monkeys, there are still considerable numbers of dopaminergic neurons left in the mesencephalon, implantation-induced trophic effects on host residual dopaminergic neurons have been suggested as a mechanism underlying the behavioral recovery. Gliosis around the graft is a universal finding in any implantation procedure and is probably mediated by interleukin-1 (IL-1); in addition, activated astrocytes secrete several neurotrophic factors in vitro. Therefore, the authors postulated that trophic effects from IL-1-induced gliosis may be a "final common pathway" for recovery in parkinsonian animals after implantation.

Hemiparkinsonism was induced in rats by injection of 6-hydroxydopamine either directly into the substantia nigra or into the median forebrain bundle. The substantia nigra-lesioned rats showed complete depletion of dopaminergic neurons in the substantia nigra but sparing of those in the ventral tegmental area, whereas the median forebrain bundle-lesioned animals had depletion of dopaminergic cells in the substantia nigra and the ventral tegmental area. Polymer pellets containing either slow-released IL-1 alpha and beta or placebo pellets were implanted in the caudate nucleus on the lesioned side in both groups. The rats' rotational response to amphetamine was tested weekly for 8 weeks. Selective substantia nigra-lesioned rats with implantation of IL-1 pellets had a 45% reduction in amphetamine-induced rotation, whereas placebo-implanted substantia nigra-lesioned rats had a 14% reduction in rotation. In the median forebrain bundle-lesioned group, neither IL-1 nor placebo implantation elicited any effect on turning. Immunohistochemical staining for glial fibrillary acidic protein was markedly increased surrounding the IL-1 pellets compared to the placebo pellets. In the selective substantia nigra-lesioned rats with IL-1 pellets implanted in the caudate nucleus, a considerable number of tyrosine hydroxylase immunoreactive (TH-IR) fibers were observed in the medial and middle portions of the caudate nucleus. Fewer TH-IR fibers were seen in the rats with placebo-bearing pellets.

These results suggest that neurotrophic activities mediated by IL-1 and reactive astrocytes might be a common path through which tissue trauma and some tissue transplants exert their beneficial effects in parkinsonian animals. Furthermore, most of the sprouted dopaminergic fibers induced by IL-1 in the caudate nucleus come from dopaminergic neurons in the ventral tegmental area.

KEY WORDS • Parkinson's disease • interleukin-1 • amphetamine • neurotrophic activity • brain implantation • rat

Parkinson's disease is characterized by the loss of mesencephalic dopaminergic cells. Cardinal manifestations of rigidity, bradykinesia, tremor, and loss of postural reflexes appear when more than 70% of nigral neurons are lost. The dopaminergic cell loss in the mesencephalon in idiopathic Parkinson's disease is not universal; the ventral tegmental area and the dorsal portion of the substantia nigra are far less affected than the ventral substantia nigra. The same pattern of cell loss (a more extensive loss of dopaminergic cells in the ventral substantia nigra than in the dorsal substantia nigra and ventral tegmental area) oc-
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ocurs in monkeys rendered parkinsonian by the administration of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydroxyridine. Although replacement therapy with L-dopa can relieve some symptoms in patients with idiopathic Parkinson’s disease, it does not prevent further dopaminergic cell loss in the midbrain or after the natural history of the disease. Introducing exogenous dopaminergic cells and enhancing the survival and function of residual host dopaminergic cells are potential ways to halt the progression of Parkinson’s disease.

Transplantation of adrenal medullary tissue into the caudate nucleus partially alleviates some of the parkinsonian motor deficits in rodents, nonhuman primates, and patients. The mechanisms originally thought to underlie this therapy were diffusion of dopamine from the transplanted tissue or specific reinnervation of the depleted striatum by the transplanted dopaminergic tissue. However, recent experimental results cast doubt on these hypotheses; some animals that showed behavioral recovery after adrenal medullary implantation exhibited limited survival of implanted chromaffin cells. Furthermore, tyrosine hydroxylase (TH) immunoreactive (TH-IR) fibers from the host have been found in the periphery of the graft. This increased TH-IR activity around adrenal implants has been reported in primates, rodents, and, more recently, in humans. Moreover, noncatecholaminergic tissue implants and cavitation alone in the striatum of hemiparkinsonian rats and monkeys also lead to similar functional recovery and the appearance of host TH-IR fibers. These data suggest that trophic factor-induced sprouting by host dopaminergic cells may play an important role in producing behavioral improvement.

The trophic hypothesis is also supported by the observation that fetal tissue implantation, even nondopaminergic fetal tissue, induces greater behavioral recovery than adrenal implantation or caudate trauma in parkinsonian monkeys. This may occur because fetal tissue contains more, or stimulates greater secretion of, neurotrophic factors than do mature adrenal implants. Because cavitation alone or adrenal implants lead to some long-term behavioral improvement, despite death of the adrenal graft, other factors must be involved. Amelioration of parkinsonism in rats occurs after the intracaudate implantation of microglia or activated leukocytes; thus, we hypothesized that inflammatory cells and reactive glia around the graft or trauma site may mediate a trophic effect. Furthermore, the cellular source of the graft-induced sprouted fibers in the caudate nucleus is not known. Dopaminergic neurons in the ipsilateral ventral tegmental area or the contralateral mesencephalon are possible sources.

In this experiment, we investigated whether implantation of interleukin-1 (IL-1), a cardinal chemical mediator of inflammation in the brain, can induce behavioral improvement in hemiparkinsonian rats. We also evaluated whether dopaminergic neurons in the ventral tegmental area might be the source of graft-induced sprouting in the striatum.

Materials and Methods

Eighty male Sprague-Dawley rats, each weighing between 200 and 275 g, were used in this experiment. Superior cervical ganglionectomies were performed in all animals prior to the experiment to prevent nonspecific adrenergic sprouting, which could interfere with the interpretation of TH-IR fibers. The protocol was approved by the Animal Care and Use Committee of the National Institute of Neurological Disorders and Stroke.

Lesioning With 6-Hydroxydopamine

Before use, 6-hydroxydopamine (6-OHDA, 2.0 μg/μl) was prepared with ascorbic acid (0.2 mg/ml) to prevent autooxidation; this solution was kept in a light-proof container on dry ice until needed. The animals were anesthetized with intraperitoneal ketamine (100 mg/kg) and xylazine (3 mg/kg) and placed in a Kopf stereotaxic frame with bite bar at 3.5 mm below horizontal level. A 2-mm burr hole was drilled on the right side of the skull to accommodate the injection of 6-OHDA. In 40 rats, 6-OHDA was injected into the substantia nigra at two sites with respect to the lambda and dura. The first (3-μl) injection of 6-OHDA was given 3.5 mm anterior posterior (AP), 1.9 mm mediolateral (ML), and 7.1 mm dorsoventral (DV) with the needle bevel facing laterally. The second (4-μl) injection was given 3.5 mm AP, 2.3 mm ML, and 6.8 mm DV with the needle bevel directed posteriorly. In the other 40 rats, a single 3-μl injection of 6-OHDA was made into the median forebrain bundle at coordinates −4.4 mm AP, 0.9 mm ML, and 7.5 mm DV with respect to the bregma and dura. The neurotoxin was injected at a rate of 0.1 μl/min, and the needle was left in place for 2 to 3 minutes after injection. The needle was withdrawn slowly to prevent backflow of 6-OHDA solution along the needle track. One week after lesioning, all 80 rats were injected subcutaneously with a challenge dose of amphetamine (5 mg/kg), and the rats’ rotational behavior was recorded for 90 minutes by a rotometer. The animals were allowed to recover for 2 days and then the rotational response to an oral dose of L-dopa/carbidopa (100 mg/10 mg/kg) was tested in a similar manner. Every animal was tested weekly with each drug for 3 weeks.

Animal Selection

In the selective substantia nigra-lesioned rat model, rotational response to L-dopa may spontaneously recover over an interval of 6 to 8 weeks; therefore, L-dopa rotation was used only to verify the ventral tegmental area lesion and not used as a means to evaluate the behavioral recovery after implantation. The baseline amphetamine and L-dopa rotational data were used to divide the animals into two groups. Twenty rats that turned more than seven times per minute after amphetamine administration in the group that received lesions of the substantia nigra and another 20 rats that turned more than five times per minute in the group with lesioning of the median forebrain bundle were selected.

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for further study. Each group was further separated into two subgroups: the control group was implanted with a placebo-loaded pellet (slow release of cholesterol/cellulose/lactose) into the caudate nucleus, and the experimental group was implanted with an identical pellet to which IL-1 had been added (IL-1 alpha and beta, 0.2 µg/pellet).

**Pellet Implantation**

The animals were anesthetized with ketamine and xylazine and placed in the stereotactic frame. A burr hole was drilled on the right side of the bregma about 3.0 mm from the midline, and a pipette tip, 0.3 mm in diameter, loaded with a 2-mm pellet containing IL-1 or placebo, was inserted at coordinates 0 mm AP, 3 mm ML, and 2.5 mm DV with respect to the bregma and dura. A piston was used to deliver the pellet into the caudate nucleus. After implantation, the animals underwent amphetamine-induced rotation tests weekly for 8 weeks.

**Phaseolus Valgaris-Leukoagglutinin Injection**

An anterograde tracer, Phaseolus vulgaris-leukoagglutinin (PHA-L), was used in this experiment to identify the cellular origin of the sprouted fibers in the striatum after implantation. Micropipettes with a tip diameter of 10 µm were backloaded with the PHA-L solution using a vacuum line. The pipettes were inserted into the ventral tegmental area stereotactically. The ventral tegmental area is close to the midline; thus, a vertical 15° angle was used to avoid damaging the superior sagittal sinus. After the pipettes were in place, 10 µA of positive (cathodal) current was delivered in 7-second pulses every 14 seconds over a 15-minute period using a constant-current device. The animals were allowed to survive for 5 days to permit axonal transportation.

**Immunohistochemical Staining**

At the end of the experiment, the rats were anesthetized and intracardially perfused with phosphate-buffered saline (PBS) followed by 200 ml of 10% formalin. The brains were removed and postfixed in the same solution for at least 24 hours. Coronal slices in the midbrain and the striatum were cut 50 µ thick using a vibratome. The sections were stained with antibodies against TH, glial fibrillary acidic protein (GFAP), and PHA-L using a previously described method. Bovine serum albumin was used to block endogenous peroxidase activity. The sections were incubated overnight in the primary antisera raised in rabbits (1:2000 dilution in PBS also containing 2% goat serum and 0.3% Triton X-100) at 4°C. The sections were then transferred for 1 hour to a solution of biotinylated goat anti-rabbit immunoglobulin G maintained at room temperature (using the dilution recommended by the supplier) that also contained 2% normal goat serum and 0.3% Triton X-100. After the sections were rinsed for 30 minutes in PBS containing 1% goat serum and albumin, they were placed in an avidin-biotin-peroxidase complex reagent for 1 hour. After three 5-minute rinses, sections were processed for peroxidase histochemistry. A solution containing 5 mg 3,3'-diaminobenzidine and 0.04% H2O2 in 10 ml PBS was filtered just prior to use. Sections were incubated in this solution for 1 to 5 minutes, rinsed at least three times in PBS, mounted onto the gelatinized slides, air-dried, and covered. Representative midbrain slices were then digitized on a computer image-analyzing system, and TH-IR cells in the substantia nigra and ventral tegmental area were counted on both sides.

**Results**

Selective substantia nigra-lesioned rats showed stable amphetamine (> 7 turns/min) and L-dopa (< 200 turns/90 min) rotation for 3 weeks. Rats that received median forebrain bundle lesions demonstrated 5 to 7 turns/min after amphetamine and more than 200 turns/90-minute period after L-dopa administration. No change in rotational behavior in response to amphetamine was observed in median forebrain bundle-lesioned rats that were implanted with either IL-1 or placebo pellets (Fig. 1A). A 45% decrease in amphetamine-induced rotation from the 4th to the 6th week postimplantation was observed in substantia nigra-lesioned rats that received IL-1 implants; substantia nigra-lesioned rats that were implanted with placebo pellets had a 14% reduction in amphetamine-induced rotation (Fig. 1B). The only significant effect on rotation was in the substantia nigra-lesioned rats implanted with IL-1 pellets (p < 0.05, Fig. 1C).

Brain slices from both substantia nigra- and median forebrain bundle-lesioned rats, when stained with anti-GFAP antibody, showed intense GFAP uptake in the striatum around the IL-1 pellets. Gial fibrillary acidic protein activity around the placebo pellets was observed in both groups, but it was substantially less than that in IL-1-implanted rats. In median forebrain bundle-lesioned animals, there was complete loss of TH-IR neurons in the substantia nigra and more than 95% depletion in the ventral tegmental area (Figs. 2 and 3). No TH activity could be detected in the caudate nucleus or the nucleus accumbens septi, regardless of whether the pellet contained IL-1 (Fig. 3A to D). Selective substantia nigra-lesioned rats showed complete depletion of TH-IR cells in the substantia nigra, whereas more than 80% of the TH-IR cells in the ventral tegmental area (compared with the normal side) were preserved on the lesioned side. In the striatum, the placebo-implanted substantia nigra-lesioned rats had only sparse TH-IR fibers along the ventricle on the lesioned side (Fig. 3F). The nucleus accumbens septi and olfactory tubercle, however, remained symmetrically stained. In contrast, the substantia nigra-lesioned rats...
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Imagined with IL-1 pellets demonstrated considerable TH-IR fibers in the medial and middle portions of the caudate nucleus with a decreasing gradient of intensity of TH activity from ventromedial to dorsolateral (Fig. 3H). In five substantia nigra-lesioned rats that showed behavioral recovery after IL-1 implantation, PHA-L was injected into the ventral tegmental area on the side in which the substantia nigra was completely lesioned. In three animals, PHA-L staining revealed that the injection was precisely in the ventral tegmental area target (Fig. 4B). At the center of the injection, neurons and glial cells appear to be filled with lectin, and both perikarya and dendrites were intensely labeled. On the striatum sections stained with anti-PHA-L antibody, PHA-L-positive fibers are clearly identified in the medial and middle portions of the caudate (Fig. 4C), as well as in the nucleus accumbens septi and olfactory tubercle. The distribution of PHA-L-positive fibers in the caudate correlates well with the distribution of the TH-stained fibers in neighboring slices from the same

Fig. 2. Drawings showing relevant structures seen in midbrain slices (left) and striatum slices (right). SNC = substantia nigra pars compacta; SNR = substantia nigra pars reticulata; VTA = ventral tegmental area; PN = paranigral nucleus; CC = corpus callosum; OT = olfactory tubercle; AN = nucleus accumbens; CP = caudate-putamen complex.
Brain slices stained with antibody against tyrosine hydroxylase (TH): see Fig. 2 for relevant structures. In the left column (A, C, E, and G) is a TH-stained midbrain slice from one animal in each group; in the right column (B, D, F, and H) are the TH-stained striatal slices from the same animals. A unilateral lesion of the median forebrain bundle produces complete depletion of TH immunoreactive (TH-IR) neurons in the ipsilateral substantia nigra and ventral tegmental areas (A and C). In the median forebrain bundle-lesioned group, no TH-IR activity was found in the striatum implanted with placebo (B) or with interleukin-1 (IL-1) pellets (D). In rats receiving a selective substantia nigra lesion, however, midbrain sections show a complete loss of TH-IR cells in the substantia nigra and a largely intact ventral tegmental area (E and G). Animals implanted with placebo pellets show symmetrical TH staining at the nucleus accumbens septi and olfactory tubercle, but no significant TH activity in the caudate nucleus on the lesioned side (F). In animals implanted with IL-1 pellets, considerable TH-IR fibers stain in the ventromedial and middle portions of the caudate nucleus on the lesioned side (H).
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animals (Fig. 4A), although PHA-L-positive fibers were not as dense as TH fibers.

Discussion

The results from this experiment indicate that implantation of IL-1 into the striatum induces behavioral recovery in rats that have received selective lesions of the substantia nigra. Histologically, the IL-1 pellets lead to similar astrocytic activation (increased GFAP expression) in the substantia nigra- and median forebrain bundle-lesioned groups. However, the only group in which the behavior improved, the substantia nigra-lesioned IL-1-implanted group, was also the only group with increased TH immunoreactivity in the implanted caudate nucleus. The pattern of increased TH-IR expression is quite similar to the sprouting demonstrated in previous studies after tissue implantation, suggesting that IL-1-induced gliosis may be a common path through which nondopaminergic implants exert therapeutic effects. Activated astrocytes secrete nerve growth factor (NGF)-like neurite-promoting compounds in vitro, which could induce dopaminergic sprouting in the host striatum. Interleukin-1 is a pluripotential growth-promoting factor; it is a fibroblast mitogen and can be secreted by microglia, macrophages, and lymphocytes. After brain injury, increased amounts of IL-1 are found around the lesioned site and are coupled with gliosis. Adding IL-1 to astrocyte cultures increases the cell number by 20-fold. In similar cultures, NGF messenger ribonucleic acid expression is doubled and NGF secretion triples with the addition of IL-1. These observations suggest that IL-1 is a key mediator of inflammation in the brain and that it might be a key chemical through which tissue implants exert their trophic effects on residual host dopaminergic neurons.

The traditional way to render rats parkinsonian is to unilaterally lesion the median forebrain bundle which is concentrated near the midline. This depletes neurons in the substantia nigra and ventral tegmental area on one side of the midbrain and contrasts sharply with the pattern of partial cell loss in idiopathic Parkinson’s disease and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced parkinsonism in primates, which principally affect the substantia nigra. Thus, the traditional median forebrain bundle-lesioned rat is not an ideal model with which to study graft interactions (trophism) with residual host dopaminergic neurons. The histology of our selective substantia nigra-lesioned rats closely resembles the midbrain histology seen in parkinsonian patients. Because the ventral tegmental area projections overlap those of the substantia nigra in the medial portion of the caudate nucleus, the ventral tegmental area constitutes an important potential source for dopaminergic sprouting after dopaminergic depletion occurs.

This study indicates that dopaminergic sprouting can be enhanced by introducing trophic factors into the striatum. The reactive astrocytes, induced by IL-1, are a likely source of the trophic factors that underlie the behavioral and histological recovery. Tyrosine hydroxylase immunoreactive fibers in the caudate nucleus were observed only in the selective substantia nigra-lesioned rats after IL-1 pellet implantation but not in median forebrain bundle-lesioned animals, which strongly suggests that the new dopaminergic fibers came from intact ventral tegmental area neurons.
In the substantia nigra-lesioned animals that showed significant behavioral improvement after IL-1 implantation, anterograde tracer PHA-L was introduced into the ventral tegmental area on the lesioned side by electrophoresis. Many PHA-L-positive fibers could then be seen in the medial and middle portions of the caudate nucleus. The distribution of the PHA-L-positive fibers overlapped with the distribution of TH-IR fibers present on the adjacent sections provided additional direct evidence that the sprouted fibers in the striatum originate from the ipsilateral ventral tegmental area.

References


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