Reversal of experimental parkinsonism by using selective chemical ablation of the medial globus pallidus

Daniel M. Lieberman, M.D., Marc-Etienne Corthesy, M.D., Alex Cummins, M.A., and Edward H. Oldfield, M.D.

Central Nervous System Implantation Unit, Surgical Neurology Branch, National Institute of Neurologic Disorders and Stroke, National Institutes of Health, Bethesda, Maryland

Object. Symptoms from Parkinson's disease improve after surgical ablation of the medial globus pallidus (GPM). Although, in theory, selective chemical ablation of neurons in the GPM could preserve vital structures jeopardized by surgery, the potential of this approach is limited when using traditional techniques of drug delivery. The authors examined the feasibility of convection-enhanced distribution of a neurotoxin by high-flow microinfusion to ablate the neurons of the GPM selectively and reverse experimental Parkinson's disease (akinesia, tremor, and rigidity).

Methods. Initially, to test the feasibility of this approach, the GPMs of two naive rhesus macaques were infused with kainic acid or ibotenic acid through two cannulas that had been placed using the magnetic resonance imaging-guided stereotactic technique. Two weeks later the animals were killed and their brains were examined histologically to determine the presence of neurons in the GPM and the integrity of the optic tract and the internal capsule. To examine the therapeutic potential of this paradigm, unilateral experimental Parkinson's disease was induced in six macaques by intracarotid infusion of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and their behavior was studied for 12 weeks after chemopallidotomy was performed using kainic acid (three animals) or control infusion (three animals).

Conclusions. Chemopallidotomy using kainic acid permanently reversed the stigmata of MPTP-induced parkinsonism. By contrast, the control animals exhibited a transient recovery following intrapallidal infusion and then relapsed back to their baseline state. The use of high-flow microinfusion of selectively active toxins has the potential for treatment of Parkinson's disease and, by expanding the range of approachable targets to include large nuclei, for broad applications in clinical and experimental neuroscience.

Key Words • pallidotomy • Parkinson's disease • excitotoxin • kainic acid • 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine • Macaca mulatta

PARKINSON'S disease is produced by deregulated, dysfunctional neural activity in the medial globus pallidus (GPM). Clinical studies have empirically identified the GPM as an effective target for ablation to relieve the syndrome. Despite advances in surgical technique and the use of electrophysiological monitoring to identify pallidal neurons, techniques now in use for ablation using radiofrequency or radiation are inherently not selective and jeopardize the internal capsule and the optic tract, which lie adjacent to the GPM. Therefore, the extent and location of pallidal lesions that can be made safely with those techniques are limited.

Selectively active neurotoxins such as excitotoxins provide a chemical means of destroying pallidal neurons while preserving adjacent glial cells and axonal fibers of passage that may be damaged when using nonselective techniques. However, these agents cannot be administered by traditional techniques for regional drug delivery in the human central nervous system, because those delivery techniques rely on diffusion of molecules from the point of administration. In contrast, it has recently been shown that high-flow microinfusion can be used to produce interstitial convection of fluid in the brain at relatively homogeneous concentrations and to deliver drugs efficiently to large nuclei or other brain regions.

Initially, to test the feasibility of using high-flow microinfusion of an excitotoxin to lesion a large nucleus in the primate brain, we perfused the GPM in two naive rhesus macaques with either kainic acid or ibotenic acid. Subsequently, to test the therapeutic potential of this paradigm in six monkeys that previously had been rendered unilaterally parkinsonian by intracarotid infusion of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), we compared the behavior and brain tissue characteristics in three animals infused with kainic acid with those in another three animals that received control infusions.

Materials and Methods

The housing, care, handling, procedures, and anesthesia used for the rhesus macaques in this study were in accordance with the Guidelines on the Use of Animals in Experimental Research from the National Institutes of Health and were approved by the National Institute of Neurologic Disorders and Stroke Animal Care and Use Committee.

Direct Interstitial Infusion of the GPM

Operative procedures were identical for the determination of feasibility and for examining the therapeutic potential of neurotoxin infusion. After anesthesia had been induced by intramuscular administration of ketamine (10 mg/kg) and xylazine (3 mg/kg) and intravenous administration of thiopental-sodium (2–6 mg/kg), each macaque underwent placement of a No. 4 French endotracheal tube.
FIG. 1. Photomicrographs showing that high-flow microinfusion of kainic acid selectively eliminates neurons while preserving adjacent glial cells and white matter. After the macaques were killed 2 weeks following infusion, serial sections of the GPm were stained with hematoxylin and eosin. In the opposite (uninfused) GPm (A, upper), neurons are readily distinguished from glial cells by their size, glassy cytoplasm, and pyramidal shape; whereas the kainic acid-infused GPm is completely devoid of neurons (A, lower) but contains dense gliosis. Fig. 1 (continued)
Magnetic resonance (MR) images of the brain were acquired with the macaque’s head secured in a stereotactic frame and by using gadolinium-impregnated ear and bite bars. The coordinates of the center of the GP relative to the stereotactic frame were then calculated. The animals were transported to the surgical suite, where anesthesia was maintained using isoflurane inhalation (1–2%) while temperature, blood pressure, heart rate, respiratory rate, oxygen saturation, and end-tidal carbon dioxide were monitored continuously. Each animal’s scalp was shaved and scrubbed with povidone iodine. The macaques then received intravenously administered dexamethasone (0.7 mg/kg), furosemide (5 mg/kg), mannitol (1 g/kg over 20 minutes), and cefazolin (12–20 mg/kg). With the use of MR imaging–guided stereotactic localization, infusion points were centered at the junctions of the middle and distal thirds of the pallidum in the rostral–caudal axis. A silicone infusion cannula was inserted and placed with its tip at the center of the target through an incision in the scalp and a burr hole through the skull.

After 30 minutes had elapsed, infusion of kainic acid (0.1 mg/ml phosphate-buffered saline [PBS]) was begun by using a syringe infusion pump. The infusion rate was increased from 0.2 to 0.4, 0.6, 0.8, and up to 1 μl/minute at 15-minute intervals and was maintained at 1 μl/minute until 75 μl were infused; the total infusion time was 100 minutes. Ibotenic acid (1 mg/ml PBS) was infused in an identical manner. Thirty minutes after the infusion was stopped, the cannula was withdrawn slowly and the galea and scalp were sutured closed. After an intramuscular injection of dexamethasone (0.7 mg/kg) had been administered, the macaques were observed throughout their recovery from anesthesia. Signs of pain were treated with Banamine (1.33 mg/kg given three times per day). On the same day (following surgery) all macaques resumed their routine preoperative activities.

Histological Investigation

Following infusion of kainic or ibotenic acid, the macaques were killed after 2 weeks (feasibility study, two animals) or 12 weeks (therapeutic potential study, six animals) of survival by intravenous administration of thiopental–sodium (30 mg/kg) and transcardial perfusion with chilled PBS (200 ml, −4°C) and formalin (500 ml at −4°C). Serial sections of the pallidum were stained with hematoxylin and eosin, thionin–Luxol periodic acid–Schiff, and silver. Immunocytochemical staining was performed for glial fibrillary acidic protein. The lesioned brain areas identified on the hematoxylin and eosin–stained brain sections were mapped onto a scaled atlas of the macaque brain and scanned into an image analysis system (Image 1.3; National Institutes of Health, Bethesda, MD). Lesion volumes were calculated as the sum of cross-sectional lesion areas on representative sections throughout the brains.

Induction of Unilateral Parkinsonism and Behavior Testing

For the therapeutic potential study, stable unilateral Parkinson’s disease was induced in six macaques by contralateral infusion of MPTP (0.4 mg/kg in 20 ml normal saline) into the internal carotid artery. The animals’ behavior was scored using a hemiparkinsonian rating scale, as previously described. Briefly, the animals were rated weekly throughout the study period by an unblinded investigator (D.M.L.), and all sessions were videotaped for documentation. The animals were placed in a modified Wisconsin testing apparatus with a transparent plastic front wall for observation of motor performance. The macaques were offered food treats by the investigator and their hand preference for retrieval was noted. Next the animals were given a large piece of fruit and their ability to use the affected arm was recorded. Finally, a plastic tray was mounted on the cage and food treats were placed at increasing distances from an opening in the cage. The capability of the animals to retrieve treats with their affected hands was scored.

After 1 to 2 years of stable parkinsonism after MPTP lesioning, three macaques underwent infusion of the GPm with kainic acid (19–23 μg in 190–230 μl PBS) through two cannulas. Ibotenic acid solution (1 mg/ml) was infused into the GPm of three other macaques used as control animals. In addition to routine behavioral evaluations (as described earlier), the animals were offered food treats in their home cages from a variety of angles, to determine whether a visual field deficit was present. At the end of the experiment the macaques were killed and their brains were examined histologically in the manner described earlier.

Results

Feasibility Study

To determine the feasibility of high-flow microinfusion...
to distribute a selectively active neurotoxin in a target region of the brain, we infused the GPm of one naive rhesus macaque with kainic acid (7.5 μg in 75 μl PBS delivered over 100 minutes) and the GPm of another with ibotenic acid (75 μg in 75 μl PBS). The kainic acid infusion produced a sphere occupying 180 μl of the GPm that, characteristically for an excitotoxic lesion, contained glial cells and myelinated axon tracts, but was depopulated of neurons (Fig. 1A and B). The boundary of the lesion was sharply demarcated histologically, with neurons being either completely absent or present in normal density. The distinct margin presumably reflected a sharp boundary between naive and infused brain. There were no changes in fiber density in the optic tracts, internal capsule, or GPm, as examined with luxol–periodic acid–Schiff (not shown) and silver staining (Fig. 1C–F). Before the animals were killed, the macaque treated with kainic acid exhibited no neurological deficits.

In contrast to kainic acid, the ibotenic acid infusion produced no difference in the number of medial pallidal neurons between nuclei in the infused and uninfused hemispheres.

**Therapeutic Potential Against Parkinsonism**

We tested the therapeutic potential of selective chemopallidotomy in naive Rhesus macaques that had been rendered unilaterally parkinsonian (Figs. 2–4). Three macaques underwent complete pallidotomy produced by high-flow microinfusion of kainic acid to the GPm; three others received ibotenic acid infusion and served as a control group. Because we already had demonstrated no reduction in the number of GPm neurons following its infusion, ibotenic acid solution was selected to control for osmotic and mechanical damage to the brain.

Immediately after the uncomplicated chemopallidotomy had been performed, the three macaques that received kainic acid exhibited purposeful, spontaneous use that was older succumbed to myocardial infarction, as inferred from postmortem examination. In the other two macaques, hemiparkinsonian posture and tremor gradually resolved during the week following the selective medial pallidal ablation. At 3 weeks after infusion, T2-weighted MR images revealed that the lesion produced by kainic acid incorporated most of the internal pallidum (Fig. 2 right). The distribution of the increased T2 signal coincided with the distribution of the lesion demonstrated histologically. After extensive pallidal ablation the macaques were capable of reaching out of their cages, grasping pieces of fruit, and feeding themselves with their contralateral extremity (Fig. 3 lower). Just before they were killed 12 weeks after chemopallidotomy, these animals were free of tremor, rigidity, and akinesia.

Histological examination of the GPm in the two macaques that completed the protocol revealed a dramatic (79% and 80%) reduction in neurons compared with the unlesioned contralateral side (Fig. 4) and showed no damage to the white matter fibers in the GPm, internal capsule, or optic tract (Fig. 1C–F). In one macaque neuronal cell loss was noted in the CA3 region of the hippocampus ipsilateral to the side of pallidal infusion, possibly a result of the high volume of kainic acid infused relative to the volume of the GPm. No behavioral, neurological, or physiological sequelae of this extended effect were apparent.

In contrast with the macaques that received kainic acid, the control macaques, which received ibotenic acid, only exhibited reduced akinesia for several days after the infusion (Fig. 3 upper). Within 4 to 7 days after the infusion, these animals exhibited complete and lasting recurrence of the Parkinson’s disease. Because that interval corresponds to the period of surgical edema, the relapse was probably due to recovery from the trauma of catheter placement and from the edema induced by the infusion. This is supported by the results of the histological investigation, in which these macaques showed no reduction in

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**Fig. 3.** Graph showing that chemopallidotomy reverses experimental parkinsonism in naive rhesus macaques. Three control macaques in which the GPm was infused with ibotenic acid (upper) exhibited transient improvement, but relapsed into severe Parkinson’s disease in conjunction with the expected resolution of edema related to cannula insertion and infusion. Macaques infused with kainic acid (lower) demonstrated dramatically improved arm use that was sustained throughout the 12-week study period. The initial macaque infused with kainic acid (box icons in lower graph) received a subtherapeutic dose (7.5 μg) placed in the caudal aspect of the pallidum, as verified by MR imaging. That macaque experienced a relapse 3 days after the infusion, but another treatment with a higher dose (1 μg) produced a lasting therapeutic effect. Note that one macaque that succumbed to a fatal myocardial infarction 2 days after uncomplicated kainic acid infusion is not represented because of insufficient behavioral data.
the number of medial pallidal neurons in the treated side compared to the contralateral side.

**Discussion**

Drug delivery systems such as polymer implants, slow osmotic minipumps, and intraventricular injection, which establish a high concentration gradient of a drug between its delivery point and adjacent brain and then rely on diffusion for wider distribution, are impractical for intracerebral delivery of chemical toxin because 1) uncontrolled diffusion may inadvertently damage structures adjacent to the target; 2) the magnitude of possible distribution, based on the diffusion, is generally smaller than most brain regions and single large nuclei; and 3) diffusion produces an exponential decline in drug concentration at increasing distances from the point of delivery, creating a narrow therapeutic window. In contrast, delivery and distribution by high-flow microinfusion of drugs into white and gray matter distributes molecules within the extracellular space at relatively homogeneous concentrations and on a scale that permits the treatment of large brain regions or nuclei in primates. We took advantage of these features of convection-enhanced distribution to deliver the kainic acid, which effected an excitotoxic lesion occupying most of the GPm, reversing the experimental parkinsonism without damaging the internal capsule or optic tract.

During interstitial infusion, a pump was used to generate sufficient force to drive kainate molecules in a radial direction outward from the point of delivery. In theory, the maximum radial distance from the point of infusion achievable by using this technique is established when the bulk flow velocity of particle movement and fluid flux slows to that of diffusion; before that point, molecules are distributed at a homogeneous concentration. Because kainic acid molecules interact reversibly with their receptors in the extracellular space without becoming...
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In contrast to patients of the nucleus, is nearly contiguous with the sensorimotor component in the posterior ventral aspect of the nucleus. In recent studies in humans that demonstrate a topographic organization of the GPm, in which the mechanism of this distant effect likely relates to the high volume of kainic acid infused, redistribution of kainic acid molecules in the cerebrospinal fluid, or damage resulting from an unserved seizure by the animal.

Selective chemical ablation of the GPm reverses MPTP-induced parkinsonism. The importance of this result is accentuated by recent studies in humans that demonstrate a topographic organization of the GPm, in which the sensorimotor component in the posterior ventral aspect of the nucleus is nearly contiguous with the optic tract and internal capsule. In contrast to patients undergoing traditional pallidotomy, the macaques in our study were not given dopaminergic agonists following surgery. Thus, the nearly complete reversal of severe parkinsonism exhibited by the macaques following chemopallidotomy is particularly encouraging because the behavioral recovery observed was not supplemented by medications such as exogenous dopamine. Because of distant neuronal injury associated with kainic acid infusion in the brain, it is not a consideration for clinical use. However, there are a variety of chemicals that may be used to target glutamate receptors on neurons in the GPm that do not produce the dangers of distant injury associated with kainic acid.

This strategy of directly altering the regional neurotransmitter balance by the selective destruction of pheno-

typically distinct cells has potential application in the development of new treatments for a broad spectrum of neurological diseases. In particular, some disorders now treated with systemic administration of neurotransmitter agonists or antagonists, including epilepsy, schizophrenia, and movement disorders, may be susceptible to this approach by using a neurotoxin appropriate for the target. Moreover, the capacity for selective chemical ablation of a single, large neural constituent, as shown here, should permit the generation of new experimental models for studying the physiology of the central nervous system.

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Current address for Dr. Lieberman: University of California, San Francisco, California.
Current address for Mr. Cummins: National Institute of Mental Health, National Institutes of Health, Rockville, Maryland.
Address reprint requests to: Edward H. Oldfield, M.D., Surgical Neurology Branch, 10/5D37, 9000 Rockville Pike, Bethesda, Maryland 20892. email: Oldfield@box-o.nih.gov.