Autotransplantation of the superior cervical ganglion into the brain

A possible therapy for Parkinson's disease

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The superior cervical ganglion (SCG) of rats was transplanted into their own parietal cortex. Four weeks after implantation, catecholamine histofluorescence revealed many transplanted catecholamine cells in the cortex. However, no fibers extended from the transplanted tissue to the cerebral cortex. In a second group of rats which had been pretreated with 6-hydroxydopamine (a specific neurotoxin to the catecholamine neuron), some showed extension of catecholamine fibers to the cerebral cortex.

To simulate an animal model of Parkinson's disease, MPTP (1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine) was administered to five monkeys. Two weeks after MPTP administration, dopamine terminals in the caudate nucleus disappeared. After autotransplantation of the SCG into the caudate nucleus of these monkeys, many of the transplanted SCG cells extended axons beyond the graft into the caudate nucleus. These results show that transplanted SCG cells survived well in the brain. Under special circumstances, such as a shortage of catecholamine in the brain, implanted SCG cells extended their axons into the brain. It is suggested that autotransplantation of SCG grafts may be a new therapy for Parkinson's disease.

KEY WORDS • Parkinson's disease • autotransplantation • superior cervical ganglion • methylphenyl tetrahydropyridine • monkey

It is well known that Parkinson's disease results from the loss of dopamine neurons in the substantia nigra. To supplement the dopamine deficiency in the brain, L-3,4-dihydroxyphenylalanine (L-dopa) is usually administered to patients with Parkinson's disease. Problems such as the diminution of effect, intermittent effect, or dyskinesia have occurred in patients with long-term administration of L-dopa. A therapy that could be used as a substitute for L-dopa without these phenomena would benefit patients who have been suffering from Parkinson's disease for many years. Transplantation of dopamine cells into the brain has been suggested to supplement the deficiency of dopamine in these patients. Recently, an increasing number of papers have described transplantation of embryonal nervous tissue into the brain of young animals. However, this approach is difficult as a clinical application, because of ethical and practical problems with the use of human embryonal brain. In addition, the rejection of implanted tissue may be a problem.

To circumvent these limitations, transplantation of the superior cervical ganglion (SCG) into the brain was attempted in the present study. Since the SCG is rich in cells containing norepinephrine and dopamine, it was thought that transplantation of the SCG into the brain might serve to supplement the catecholamine deficiency in Parkinson's disease. The present experiment in animal models was designed to investigate the possibility of transplanting peripheral catecholaminergic cells to the brain as a therapy for Parkinson's disease.

Materials and Methods

Catecholamine Histofluorescence Studies of SCG

The SCG of five Sprague-Dawley rats, each weighing 250 to 300 gm, and one Macaca fuscata monkey weighing 5 kg was investigated by catecholamine histofluorescence. The method for catecholamine histofluorescence has been described in detail elsewhere. Briefly, the animals were perfused with 300 ml (for the
rats) or 5000 ml (for the monkey) of 2% glyoxylic acid solution containing 10% sucrose (pH 7.4, 0.1 M phosphate buffered) at 2° to 4°C. The SCG was removed under an operating microscope, and the tissue was cut in 16-μm sections by a cryostat. The sections were immersed in the perfusate for 2 to 3 minutes, then dried by a commercial hair dryer for 1 to 2 minutes and heated in an oven at 95°C for 7 minutes. The sections were coverslipped using liquid paraffin, and observed under a fluorescence microscope. The monkey’s substantia nigra and striatum were also removed and processed for catecholamine histofluorescence as described above.

**Transplantation of SCG into Rat Brain**

Sixty rats, each weighing 250 to 300 gm, were used in this study. Of these, 20 rats were anesthetized with a gas mixture of 1% to 1.5% Fluothane (halothane) in about 40% oxygen and 60% nitrogen oxide. The SCG was carefully removed under an operating microscope and cut into three pieces, which were kept in sterile phosphate-buffered saline (PBS, pH 7.4) at 2° to 4°C. The skull of the same animal was opened and the right parietal cortex was explored. A small area (5 × 5 mm) of the cerebral cortex was removed by suction to make a small cavity. After the bleeding was stopped, the three pieces of SCG were put into the cavity. Four weeks after transplantation, the animals were sacrificed and the brains processed for catecholamine histofluorescence as described above. In another group of 20 rats, the number of surviving cells was counted under catecholamine histofluorescence at various time points after transplantation.

A third group of 20 rats was pretreated with 6-hydroxydopamine (6-OHDA, a specific neurotoxin of catecholamine neurons). Specifically, 5 μl of 6-OHDA (4 mM with 0.2% ascorbic acid) was injected into the right parietal cortex over a 10-minute period. After 1 week, the SCG of the animal was transplanted into the previously injected site of the parietal cortex. Between 1 day and 4 weeks after transplantation the brains were processed for catecholamine histofluorescence at various time points after transplantation.

**Transplantation of SCG Into Parkinsonian Monkeys**

Five monkeys (*Macaca fuscata*), each weighing 5 to 10 kg, were used for this study. In order to simulate Parkinson's disease, 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP, 0.5 mg/kg/day) was intravenously injected into these monkeys six times over a 12-day period. After a total MPTP dose of 3 mg/kg had been injected, the monkeys manifested Parkinson's syndrome: bradykinesia, muscle rigidity, and occasional tremors. They were anesthetized with fluothane and the SCG was removed under an operating microscope. The fibrous capsule of the SCG was carefully removed and cut into small pieces (1 to 2 mm in diameter). These pieces were maintained in sterile PBS. Each monkey’s head was fixed in a stereotaxic apparatus and a skin incision and burr hole for transplantation were made. Through a glass cannula (1.0 mm in inner diameter and 1.5 mm in outer diameter) with an inner syringe made of stainless steel, the small pieces of SCG were transplanted into the caudate nucleus (A:22, L:2, D:15, according to the Atlas of Kusama and Mabuchi). Two weeks after transplantation, the monkeys were sacrificed and the brains processed for catecholamine histofluorescence as described above.

**Results**

**Catecholamine Histofluorescence of SCG**

In the rat and monkey SCG’s, many large ganglion cells (referred to below as “principal ganglion cells,” Fig. 1 arrow) displayed green catecholamine fluorescence. These large ganglion cells were intermingled with small cells which also showed strong green fluorescence (referred to below as small intensely fluorescent (SIF) cells, Fig. 1 arrowheads). A count of the number of these two types of catecholamine cells in the SCG from five rats yielded a mean (± standard deviation) of 14,850 ± 1237 principal ganglion cells per SCG and 860 ± 89 SIF cells per SCG. Strongly fluorescent catecholaminergic fibers were observed among these catecholamine cells.

**Transplantation of SCG Into Rat Cerebral Cortex**

Four weeks after transplantation of the SCG, small blocks of the transplanted SCG survived in 17 (85%) of the 20 rats. In these blocks, principal ganglion cells and SIF cells were seen (Fig. 2 left). The number of surviving catecholamine cells was counted after transplantation. The percentage of original catecholamine cells surviving was 26.7% ± 8.5% at 1 day, 17.5% ± 3.5% at 3 days, 10.0% ± 6.0% at 7 days, and 8.2% ±
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5.0% at 14 days after transplantation (Fig. 3). Surviving catecholamine cells giving off many catecholamine fluorescent fibers were seen in the SCG transplants, but no fibers extended to the cerebral cortex (Fig. 2 left).

In 19 (95%) of 20 rats pretreated with 6-OHDA, blocks of the SCG survived with strongly fluorescent large catecholamine fibers. Four (20%) of these rats revealed an outgrowth of catecholamine fibers from the transplant into the cerebral cortex where central catecholamine fibers had disappeared (Fig. 2 right). This contrasted with the findings in the 20 rats not treated with 6-OHDA, none of which had outgrowth of fibers into the brain (Table 1).

**Transplantation of SCG Into Parkinsonian Monkeys**

One week after the last injection of MPTP (total 3 mg/kg), the monkeys revealed bradykinesia and muscle rigidity. In these monkeys no fluorescent catecholamine cells were observed in the substantia nigra. The caudate nucleus of a normal monkey revealed many fine varicosities of catecholamine terminals (probably dopaminergic), while in the caudate nucleus of the MPTP-injected monkeys only a few catecholamine terminals were observed. Two weeks after SCG transplantation, the catecholamine cell bodies were found to have survived well in the transplants around the caudate nucleus. Many catecholamine fibers grew from the transplant into the caudate nucleus (Fig. 4).

**Discussion**

In the SCG, there are two kinds of catecholamine cells: principal ganglion cells and SIF cells. It has been reported that the principal ganglion cells contain noradrenaline and SIF cells contain dopamine, although this proposition remains controversial. In Parkinson's disease, besides dopamine deficiency in the caudate nucleus, many fine varicosities of catecholamine terminals (probably dopaminergic) were observed in the caudate nucleus of the MPTP-injected monkeys.

**TABLE 1**

<table>
<thead>
<tr>
<th>Animal Group*</th>
<th>No. of Rats</th>
<th>Transplant Survival Rate</th>
<th>Nerve Fiber Extension</th>
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<tbody>
<tr>
<td></td>
<td>No. Percent</td>
<td>No. Percent</td>
<td></td>
</tr>
<tr>
<td>without 6-OHDA pretreatment</td>
<td>20 17 85</td>
<td>0 0</td>
<td></td>
</tr>
<tr>
<td>with 6-OHDA pretreatment</td>
<td>20 19 95</td>
<td>4 20</td>
<td></td>
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*6-OHDA = 6-hydroxydopamine.

**Fig. 3.** Graph showing the percentage of surviving neurons after transplantation. The surviving catecholamine cells abruptly decreased to 26.7% ± 8.5% (mean ± standard error of the mean) 1 day after transplantation and continued to decrease gradually until 2 weeks.
Fig. 4. Histofluorescence study of the striatum in a monkey after transplantation of the superior cervical ganglion. Many catecholamine fibers (arrow) are growing out into the striatum. T = transplant. × 120.

After SCG transplantation in rats, the percentage of original catecholamine cells that survived decreased abruptly (26.7% at 24 hours after transplantation and 8% at 2 weeks after). Zhou, et al., studied the number of cells in SCG transplants in relation to time, employing Nissl staining of ganglion cells. The number of SCG cells abruptly decreased 1 day after transplantation, and continued to decrease up to 4 weeks. Although the number of SCG cells decreased to approximately 10% after transplantation in this study, a large number of catecholamine fibers were observed in the transplants. In tissue culture, SCG cells regenered and extended their axons at 0.1 mm/24 hrs, suggesting considerable regenerative capacity; therefore, the SCG may be quite suitable for transplantation to supplement catecholamines in the parkinsonian brain. Taking advantage of this powerful regrowth of peripheral nerve fibers, we grafted peripheral catecholamine neurons in the SCG rather than central catecholamine neurons. In the present study, SCG cells were easily transplanted into the brain. However, transplanted catecholamine cells seldom sent their axons to the brain parenchyma where the central catecholamine neuron system was intact. When central catecholamine fibers were destroyed by 6-OHDA, the transplanted cells extended their axons into the brain. In the monkeys whose catecholamine neurons were depleted by MPTP administration, the transplanted SCG cells survived and extended their axon fibers into the caudate nucleus. Two hypotheses are suggested for this process. In the first, the lack of central catecholamine induces the outgrowth of peripheral catecholamine fibers from the transplant into the brain. In the brain with sufficient catecholamines, there is no need for peripheral catecholamine fibers to extend into the brain. The second hypothesis suggests that some trophic factors may be involved in this phenomenon. Destruction of central catecholamine fibers can induce such trophic factors. Björklund and Stenevi reported that transplanted SCG fibers grew into the hippocampus if cholinergic input from the septal area was destroyed mechanically. They hypothesized that some trophic factors were taken up by cholinergic nerve terminals in the hippocampus and were transported retrogradely to the septal area. Destruction of the cholinergic septohippocampal pathway caused accumulation of trophic factors, which led to induction of peripheral catecholamine fibers into the hippocampus. In the present study, destruction of central catecholamine fibers in the brain may have resulted in accumulation of such trophic factors which may have induced regrowth of peripheral catecholamine fibers.

Kodama, et al., reported complications of superior cervical sympathectomy in 40 patients with subarachnoid hemorrhage. These patients experienced Horner's sign, transient mild hypertension, nasal obstruction, and abnormal salivation, none of which seriously bothered the patients. Therefore, superior cervical sympathectomy can be performed without any serious clinical impairment.

The present experiment demonstrated the possibility of SCG transplantation to the parkinsonian brain. However, we do not know whether transplanted SCG cells actually make contact with target cells in the caudate nucleus. Backlund, et al., and Madrazo, et al., transplanted human adrenal medulla into the brain of patients with Parkinson's disease and reported an improvement in the patients' condition. However,
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it is unlikely that matured adrenal chromaffin cells will transform into nerve cells in the brain after transplantation. For clinical application, SCG catecholamine cells should be more suitable for transplantation than adult chromaffin cells.

References


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