Neuron, Vol. 20, 169–172, February, 1998, Copyright ©1998 by Cell Press

Auto Transplants for Parkinson's Disease?

Minireview

Arnon Rosenthal
Genentech, Inc.
Department of Neuroscience
South San Francisco, California 94080

The Disease

Parkinson's disease is the third most common neurodegenerative disorder, with a prevalence of approximately 1% of the population over 65. Affected individuals suffer from debilitating deficiencies in motor functions that manifest in (1) rhythmic tremors at rest, (2) inability to initiate (akinesia) or complete (bradykinesia) routine movements, (3) muscle rigidity that leads to jerked motions, (4) postural instability, and (5) lack of facial expression. The disease is predominantly nonfamilial and does not show gender or racial bias. It is invariably progressive and 5–10 years after onset the worst patients are left bedridden and die of infections or secondary complications.

The clinical symptoms are caused by a selective loss of pigmented dopamine-producing neurons from the substantia nigra in the midbrain and a consequent decrease in dopamine at the innervation targets of these neurons, which include the striatum, cortex, and nucleus accumbens.

The cause of cell death in Parkinson's disease is unknown. Viral infections, environmental toxins, and oxidative stress induced by dopamine metabolites, are all suspected. In contrast, the consequence of dopaminergic neuron loss for the neural circuits that control movement is well understood. The dopaminergic neurons are an integral part of the basal ganglia, a group of forebrain nuclei that play an important role in motor control. In their absence, excessive inhibitory stimuli are sent from the basal ganglia through the globus pallidus to the thalamus, leading to a decrease in motor cortex activity and to the negative symptoms of Parkinson's disease: akinesia, bradykinesia, and rigidity. In addition, the loss of a feedback loop between the nigral dopaminergic neurons and the thalamus leads to the release of spontaneous periodic impulses in the thalamus, which are responsible in part for the characteristic tremors (Figure 1) (Côté and Crutcher, 1991).

What Can Be Done?

Over the years, several therapeutic approaches have been developed to counteract or compensate for the neural or chemical deficiencies that underlie Parkinson's disease (Table 1, Figure 1). The most prevalent among these is intravenous injection of L-DOPA, which was pioneered in the 1950s. L-DOPA is a precursor for dopamine; it crosses the blood–brain barrier and is most likely taken up by the remaining dopaminergic neurons and nerve terminals, where it is converted to dopamine. L-DOPA compensates for the reduction in the level of endogenous dopamine, increases the levels of dopamine in the striatum, and leads to a reversal or amelioration of akinesia, bradykinesia, and rigidity (Birkmayer and Hornykiewicz, 1976). Unfortunately, it is not effective in reducing tremors, nor does it slow the disease progression. After several years of treatment, L-DOPA produces severe side effects and is no longer efficacious, possibly because the number of endogenous dopaminergic neurons that can utilize it becomes too low.

Thus, in parallel to L-DOPA treatment, surgical lesions in the globus pallidus (pallidotomy) have been tried. Pallidotomy was practiced prior to the development of L-DOPA, discarded, and recently regained acceptance (Lang et al., 1997). The rationale behind pallidotomy is easy to understand. Since in the absence of dopaminergic inputs the globus pallidus is hyperactive, selective lesions will weaken its activity and reduce it to normal levels. Similar rationale also led to the usage of high-frequency (inhibitory) electrical stimulation in the subthalamic nucleus. The subthalamic nucleus normally stimulates the globus pallidus, and its inhibition helps reduce globus pallidus activity (Figure 1).

Although pallidotomy and electrical stimulation show promise in reducing akinesia and bradykinesia—especially akinesia that is induced by L-DOPA in advanced Parkinson's patients—they are not consistently effective in reducing tremors. In addition, many symptoms recur after only a few years. Thus, a third therapeutic approach was called for—transplantation (Yurek and Sladek, 1990). The rationale behind transplantation for Parkinson's disease is again straightforward: the idea is to replace lost dopaminergic neurons with a graft of new embryonic neurons. Transplantation of fetal dopaminergic neurons into rodent models of Parkinson's disease was pioneered in the early 1980s (Björklund and Stenevi, 1979; Perlow et al., 1979). In these initial experiments, approximately 150,000 ventral midbrain neural progenitors were transplanted, of which 10,000 were

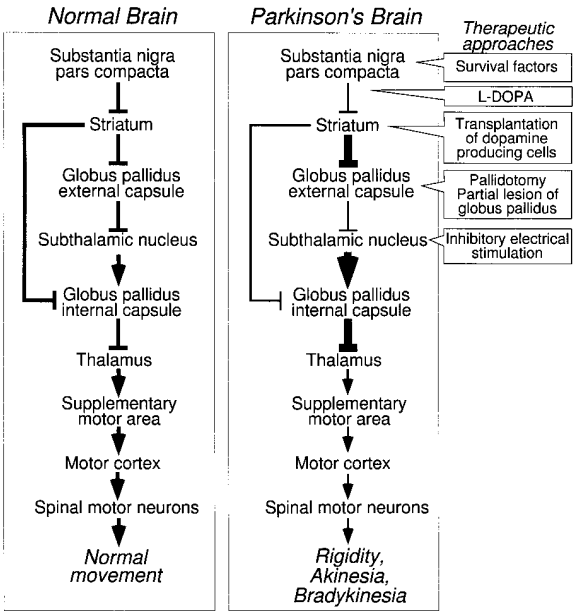

Figure 1. Neuronal Circuits in Normal and Parkinson's Brain

A simplified and partial description of neural connections affected by midbrain dopaminergic neurons in normal and Parkinson's brain and of the therapeutic approaches (after Côté and Crutcher, 1991).

Table 1. Therapeutic Approaches to Parkinson's Disease

Therapeutic Approach	Rationale	Limitation
L-DOPA	enhance dopamine production	not effective in late stages PD; does not prevent disease progression; does not prevent tremors
Pallidotomy/partial surgical lesion in globus pallidus	decrease the excessive inhibitory effect of globus pallidus on the thalamus	no consistent reversal of tremors; akinesia and bradykinesia may reoccur
Survival factors	prevent degeneration of DA neurons	difficult to deliver not tried in human
Transplantation of DA-producing cells	replace missing DA neurons	no adequate cell source

dopaminergic neurons. Unfortunately, due to the low oxygen tension or to the absence of appropriate survival factors, only 1%–10% of the grafted cells (100–1000 dopaminergic neurons) survived more than a few days. Compared to the substantia nigra of a normal rat, which contains ~30,000 dopaminergic neurons, the number of stably grafted neurons was quite low. Nevertheless, it was sufficient to restore some of the motor deficits in the rodent Parkinsonian model and to justify clinical trials in human.

Human clinical trials using dopaminergic neurons from aborted human fetuses were conducted in the late 1980s (Lindvall et al., 1988; Madrazo et al., 1988). These trials revealed a dramatic improvement in motor function, a decreased dependence on L-DOPA, and an increase in the levels of striatal dopamine in some patients. Other patients, however, showed no benefits and continued to decline. As in the animal models, the variability among human transplant recipients was subsequently shown to result from the poor survival rate of the transplanted cells. The amount of dopamine produced by the few surviving neurons was often not sufficient to ameliorate the motor deficits. As a consequence of these findings the number of transplanted cells was significantly increased. Today, up to 500,000 midbrain precursors, containing ~30,000 dopaminergic neurons (equivalent to the number of dopaminergic neurons in the entire rat substantia nigra) are being used in the rodent Parkinson's model (with the aid of antioxidants, the percentage of surviving cells has been increased to 20% or 6000 dopaminergic neurons; A. Björklund, personal communication). Likewise, it is now acceptable to use cells from up to 10 human fetuses for a single graft into a Parkinson's patient. Although this increase in cell number has led to a more consistent and favorable clinical outcome, it nonetheless renders this type of transplantation therapy impractical as a routine treatment.

The Carotid Body

Given the potential benefit of transplantation, alternative sources of cells that can provide dopamine are constantly being explored (Figure 1, Table 2). For example, adrenal medulla cells were tested in animal models and in humans (Yurek and Sladek, 1990). However, only 1/1000 of these cells survived the striatal environment for more than a few days. Moreover, although adrenal medulla cells make dopamine, they do not secrete it efficiently and therefore their therapeutic benefit is poor. Xenografts of porcine dopaminergic neurons were also successfully grafted in animal models, but the risk of interspecies viral infection may prohibit their usage in the clinic (Galpern et al., 1996). Finally, attempts are being made to recapitulate the ontogeny of dopaminergic neurons (Hynes et al., 1995) and to produce them in large amounts in a test tube. However, these studies are in the early experimental phases. Now, in this issue of *Neuron*, Dr. López-Barneo and colleagues (Espejo et al., 1998) describe another source of cells for transplantation, the glomus cells of the carotid bodies.

The carotid bodies in human are 5 mm long nodules located near the carotid sinus. They are estimated to be composed of at least 100,000 epithelial "glomus" (type I) cells and 4,000–8,000 support or sheath (type II) cells (J. López-Barneo, personal communication), and their function is to monitor changes in blood levels of O_2, CO_2, and H^+ brought about by physiological stressors such as hypoxia, high altitude, and exercise. Changes in blood gases, detected by the carotid bodies, are then reported to the medulla through the carotid sinus nerve, a component of the IX cranial nerve, and lead to an alteration in breathing that allows adequate gas exchange to meet the body's metabolic demands (Berger and Hornbein, 1989). Interestingly, the glomus cells, whose embryonic origin is the neural crest, have some neuronal-like characters and are rich in dopamine. Whereas neurons will degenerate and die at the low oxygen tensions characteristic of the transplant environment, glomus cells thrive in hypoxic conditions, producing and secreting even greater amounts of dopamine.

To test the efficacy of glomus cell grafts in a model of Parkinson's disease, López-Barneo's group transplanted them into one side of the rat substantia nigra, which had been destroyed by injection of the neurotoxin 6-Hydroxydopamine (Schwarting and Huston, 1996). Prior to transplantation, these rats had a reduction of >90% in the number of dopaminergic neurons in the lesioned side, and a similar decrease in the amount of dopamine in the ipsilateral striatum. These rats exhibited significant abnormalities in motor function including: (1) spontaneous rotation toward the lesioned side that was accentuated following treatment with amphetamine (amphetamine promotes flux reversal by plasma membrane monamine transporters, thereby releasing dopamine); (2) a 50% decrease in free locomotion that may reflect an inability to initiate movement (akinesia); (3) an increased response time in the whisker touch test, a

Table 2. Sources of Transplanted Cells

Potential Sources	Advantage	Limitations
Human fetal DA neurons	innervate the striatum; showed efficacy	no adequate source; low survival rate; possible immune response
Human fetal DA generated in vitro	abundant source	not achieved yet
Porcine DA neurons	abundant source	intraspecies viral infections; possible immune response
Dopamine-producing fibroblasts	abundant source	no innervation; no regulated secretion of dopamine
Adrenal medullary cells	abundant source	no long-term survival; no dopamine secretion
Carotid body autotransplants	no immune response; survive well in hypoxic condition; good innervation; may produce survival factors; induce rapid recovery in an animal model	small number of cells; efficacy in reversing akinesia not demonstrated

measure of sensorimotor orientation; and (4) asymmetry in the direction of movement along the cage wall, a measure of sensorimotor neglect.

Of the 400–600 glomus cells that were grafted (rats have 2,000–3,000 glomus cells per carotid body), over 60% survived for the entire duration of the experiments, which is a significantly better survival rate than for adrenal medulla cells or fetal dopaminergic neurons. Moreover, three months following the transplantation, some of the glomus cells extended through the host tissue fibers with varicose presynaptic-like structures, allowing regulated and widespread release of dopamine.

The second piece of good news is that the lesioned animals showed a rapid recovery from biochemical and motor deficits. Dopamine levels in the transplanted side increased from 11% to 65% of the contralateral striatum; the animals no longer exhibited significant spontaneous, or amphetamine-induced rotation; and their scores improved in two of the three sensorimotor tests.

The significant increase in striatal dopamine following transplantation of only 400–600 glomus cells is surprising given the fact that the rat striatum is normally innervated by ~30,000 dopaminergic neurons. This finding may suggest that glomus cells produce a lot more dopamine than the endogenous dopaminergic neurons (comparison of reported dopamine contents in glomus cells and dopaminergic neurons from different species indeed suggest that glomus cells may contain 3- to 45-fold more dopamine; J. López-Barneo, personal communication). Alternatively, since the neurons responsible for dopamine re-uptake have been lesioned, the high levels of dopamine may reflect its accumulation in the extracellular environment rather than an elevated production capacity of the glomus cells. Additional measurements of dopamine content in glomus cells and dopaminergic neurons in a single species will have to be performed in order to distinguish between these two possibilities.

The improvement in two sensorimotor tests is also quite unexpected given the low number of transplanted cells. Previous studies with fetal dopaminergic neurons have suggested that 400 neurons can elicit recovery in amphetamine-induced rotation but not in spontaneous sensorimotor tests. In these experiments, measurable recovery in sensorimotor tests was achieved only in the presence of 1,000–2,000 transplanted neurons. The finding that 400 glomus cells could improve sensorimotor tests may again indicate that they make and secrete more dopamine than dopaminergic neurons and are therefore more efficient in rescuing motor deficits. Alternatively, differences in the experimental paradigms may be responsible for the apparent discrepancies. Side-by-side comparison of these two cell types will have to be conducted to resolve this issue.

Lesioned animals that received the glomus cell grafts did not improve in the test of distance traveled in an open field, indicating that their ability to initiate and execute movements is still impaired. Reversal of this symptom, however, may require transplantation into the nucleus accumbens, a distinct innervation target of dopaminergic neurons, which lies outside of the basal ganglia. López-Barneo and colleagues did not yet attempt this.

An additional benefit of the carotid body grafts lies in their potential ability to induce arborization of the endogenous dopaminergic terminals. Such sprouting of the resident dopaminergic neurons, which is not observed with transplants of fetal neurons, may suggest that the carotid cells secrete, in addition to dopamine, a survival or a sprouting factor for dopaminergic neurons. A survival factor could greatly enhance the therapeutic potential of carotid body cells since it may be able to protect endogenous dopaminergic neurons from further degeneration. In fact, part of the observed improvement in the sensorimotor test could be attributed to sprouting of the endogenous dopaminergic neurons.

Although the carotid bodies are necessary for adequate ventilation in response to low oxygen, removal of one carotid body is not detrimental. Thus, another advantage of the carotid bodies is that they could in principle be used for autotransplantation, providing a ready source of cells and eliminating the need for immune suppression, which is now used as a precautionary measure with the fetal grafts.

The success of carotid body transplants in an animal model for Parkinson's disease obviously points to their potential use for auto transplants in humans. However, before this is attempted, several fundamental issues need to be addressed. Most important is the issue of cell number. The limited experience with fetal dopaminergic transplants into human suggests that successful grafts

may have to contain ~100,000–150,000 neurons (healthy human substantia nigra contains ~1,000,000 dopaminergic neurons). Would 60,000–100,000 glomus cells be able to do the same job? Second, beneficial effect on the more debilitating and difficult-to-reverse symptoms of Parkinson's disease, akinesia and postural balance, will have to be demonstrated. Finally, more information on the ability of glomus cells to secrete neurotrophic factors, and on the possible beneficial effects of the neuropeptides (e.g., enkephalins, VIP) and neurotransmitters (e.g., acetylcholine) that these cells produce, will have to be gathered.

Even if a single carotid body is not a sufficient cell source, the relative ease of purifying glomus cells and their abundance in the carotid body may allow the generation of useful glomus cell lines for grafting purposes. In addition, studies on the mechanisms that support the survival of glomus cells in the striatum may eventually help in improving the survival rate of transplanted primary dopaminergic neurons.

Carotid body transplants have been tried before but with less success (Bing et al., 1988). The difference appears to lie in the way the grafts were prepared in the two studies. Whereas previous investigators use dispersed cells, López-Barneo and his colleagues used cell aggregates, which probably allow better recovery and the retention of the glomus cell phenotype.

For lack of an adequate cell source, transplantation has not been a widely adopted therapeutic approach. Although it is not the ideal therapy for Parkinson's disease, it does provide significant benefits. The glomus cell grafts, which appear to provide an enriched source of dopamine-producing cells that survive well, may change this situation.

Selected Reading

Berger, A.J., and Hornbein, T.J. (1989). In Textbook of Physiology, H.D. Patton, A.F. Fuchs, B. Hille, A.M. Scher, and R. Steiner, eds. (Philadelphia: W. B. Saunders Co.), pp. 1026–1045.

Bing, G.Y., Notter, M.F., Hansen, J.T., and Gash, D.M. (1988). Brain Res. Bull. *20*, 399–406.

Birkmayer, W., and Hornykiewicz, O., (1976). Advances in Parkinsonism: Biochemistry, Physiology, Treatment, Fifth International Symposium on Parkinson's Disease Vienna. (Basel: Roche).

Björklund, A., and Stenevi, U. (1979). Brain Res. *177*, 555–560.

Côté, l., and Crutcher, M.D. (1991). In Principles of Neural Science, E.R. Kandel, J.H. Schwartz, and T.M. Jessell, eds. (New York: Elsevier), pp. 647–659.

Espejo, E.F., Montoro, R.J., Armengol, J.A., and López-Barneo, J. (1998). Neuron, this issue, *20*, 197–206.

Galpern, W.R., Burns, L.H., Deacon, T.W., Dinsmore, J., and Isacson, O. (1996). Exp. Neurol. *140*, 1–13.

Hynes, M., Poulsen, K., Tessier-Lavigne, M., and Rosenthal, A. (1995). Cell *80*, 95–101.

Lang, A.E., Lozano, A.M., Montgomery, E., Duff, J., Tasker, R., and Hutchinson, W. (1997). N. Engl. J. Med. *337*, 1036–1042.

Lindvall, O., Rehncrona, S., Gustavii, B., Brundin, P., Astedt, B., Widner, H., Lindholm, T., Björklund, A., Leenders, K.L., Rothwell, J.C., et al. (1988). Lancet *2*, 1483–1484.

Madrazo, I., Leon, V., Torres, C., Aguilera, M.C., Varela, G., Alvarez, F., Fraga, A., Drucker-Colin, R., Ostrosky, F., Skurovich, M., et al. (1988). N. Engl. J. Med. *318*, 51.

Perlow, M.J., Freed, W.J., Hoffer, B.J., Seiger, A., Olson, L., and Wyatt, R.J. (1979). Science *204*, 643–647.

Schwarting, R.K.W., and Huston, J.P. (1996). Prog. Neurobiol. *50*, 275–331.

Yurek, D.M., and Sladek, J.R. (1990). Annu. Rev. Neurosci. *13*, 415–440.