Glial cell line–derived neurotrophic factor–supplemented hibernation of fetal ventral mesencephalic neurons for transplantation in Parkinson disease: long-term storage

ADAM O. HEBB, M.D., KARI HEBB, B.Sc., ARUN C. RAMACHANDRAN, A.D.M.SCI., AND IVAR MENDEZ, M.D., PH.D.

Departments of Anatomy and Neurobiology, and Surgery (Division of Neurosurgery), Faculty of Medicine, Dalhousie University, Nova Scotia, Canada

Object. Investigation of fetal dopaminergic tissue transplantation is being conducted in animal models and clinical trials as a potential treatment for advanced Parkinson disease (PD). Because the availability of fetal tissue is limited, however, the duration of its storage prior to transplantation is a key practical issue. Longer storage times may enable fetal tissue obtained over several days to be pooled together for transplantation in a recipient. Glial cell line–derived neurotrophic factor (GDNF) has been shown to improve survival of stored human dopaminergic tissue prior to transplantation. The objective of this study was to evaluate GDNF-supplemented hibernation of fetal dopaminergic tissue for extended periods of 6 to 15 days.

Methods. A total of 27 rat ventral mesencephalons (VMs) were obtained in gestation Day 14 rat fetuses, and three were cultured immediately (fresh-culture control group). The remaining 24 VMs were divided sagittally along the midline to form 48 equal pieces of hemimesencephalons. Twenty-four pieces were stored with GDNF-supplemented hibernation medium for 6, 9, 12, or 15 days, and the 24 “partner” hemimesencephalons were stored in control hibernation medium for the same periods of time. Tissue was cultured for 48 hours and processed for tyrosine hydroxylase (TH) immunoreactivity and cresyl violet. Cell counts for all cultures and percentage of TH-immunoreactive cells were obtained. The percentage of TH-positive cells for the fresh control group was $6.3 \pm 0.5\%$; that measured in cultures derived from tissue hibernated in GDNF-supplemented medium was significantly increased at 6 and 9 days posthibernation compared with the fresh-culture control group and the partner groups stored in hibernation medium only. No significant increase in percentage of TH-immunoreactive cells was observed in the 12- and 15-day hibernation groups.

Conclusions. In summary the authors found that fetal dopaminergic tissue can safely be stored up to 9 days in GDNF-supplemented hibernation medium. Furthermore the percentage of TH-immunoreactive cells is significantly increased after 6 and 9 days of storage in this medium, improving the yield of TH-positive cells prior to transplantation. These observations may have important clinical implications for collecting fetal dopaminergic cells and improving their survival after transplantation.

KEY WORDS • glial cell line–derived neurotrophic factor • neural implantation • transplantation • Parkinson disease

Tissue grafts of fetal dopaminergic neurons have been investigated in animal studies^{2,3,13,34–37,49} and clinical trials for use as a potential treatment for advanced PD.^{8–10,20,21,26,29,31–33,58} Although the results reported in clinical studies have demonstrated graft survival and reinnervation of the putamen in human hosts,^{8,22,23} fetal tissue graft implantation in patients with PD has not reached a level of clinical efficacy that would justify its use as a routine treatment. A major disadvantage of fetal tissue is its limited supply. With current transplant methods, only 1 to 20% of the implanted dopaminergic neurons survive. To achieve useful functional recovery following transplantation, a sufficient amount of fetal mesencephalic tissue must be

obtained so that the final number of surviving grafted dopaminergic neurons would total approximately 100,000 to 150,000 cells. In practice this involves deriving tissue from three to four fetuses per side per patient. Given the limited availability of fetal tissue, this is very difficult to achieve unless tissue can be pooled from specimens collected sequentially over a number of days. Consequently, tissue storage methods that maintain cell survival over the days preceding transplantation would prove very useful.

Tissue storage strategies such as cryopreservation^{11,42} and cool storage^{12,39,43,44} help to increase tissue availability by enabling the pooling of tissue obtained in several fetuses. Cryopreservation, or freeze storage, of human VM tissue, however, has produced poor graft-related results, failing to induce functional recovery and producing poor graft volumes in the rat model of PD.¹¹ Cool storage/hibernation at 4 to 8°C has previously been successful for periods of up to 2 to 3 days, but storage for extended peri-

Abbreviations used in this paper: DMEM = Dulbecco modified eagle medium; DNase = deoxyribonuclease; GDNF = glial cell line–derived neurotrophic factor; PD = Parkinson disease; TH = tyrosine hydroxylase; VM = ventral mesencephalon.

ods (≥ 5 days) causes reduced graft volumes¹² and decreased TH-positive cell populations.^{39,43}

To increase survival of TH-positive dopaminergic neurons in cool storage, we have supplemented our hibernation medium with GDNF.^{1,30,32,33} Both GDNF and a number of related factors are a newly characterized group of trophic factors related to the transforming growth factor- β superfamily that signal through a RET receptor tyrosine kinase.^{6,16,54-56} Glial cell line-derived neurotrophic factor was initially isolated as a trophic factor for midbrain dopaminergic neurons.²⁷ It has been shown to increase TH-positive cell survival and fiber outgrowth when infused directly at the graft site; additionally it protects dopaminergic cells from neurotoxins such as 6-hydroxydopamine, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, and 1-methyl-4-phenylpyridine.^{7,14,19,24,45,47,48,52,53,59,60} Furthermore, GDNF has been implicated in the natural development of the nigrostriatal pathway,^{25,51} and it promotes expression of the typical midbrain dopaminergic neuron morphology in mesencephalon-derived progenitor cells.⁴⁰

In our studies of GDNF, we found that cool storage in GDNF-supplemented hibernation medium improves cell survival, fiber outgrowth, and promotes faster recovery in the rat model of PD. We have also shown that GDNF supplementation of hibernation medium protects human fetal dopaminergic neurons in cool storage, and we have applied this strategy to storage of VM tissue for 6 days prior to transplantation in patients with PD.^{32,33}

The objective of the present study was to investigate the efficacy of cool storage of fetal VM tissue in GDNF-supplemented hibernation medium beyond 6 days without decreased dopaminergic cell viability.

MATERIALS AND METHODS

Tissue Collection and Hibernation

Ventral mesencephalons were dissected in 27 Day 14 rat fetuses. Three of these 27 VMs were placed in culture immediately as fresh controls. The remaining 24 samples were sectioned sagittally along their midlines to form 48 equal pieces of hemimesencephalons. Half of these were placed in 6-, 9-, 12-, and 15-day hibernation groups that received GDNF-supplemented hibernation medium, and the other half were placed in 6-, 9-, 12-, and 15-day hibernation groups that received hibernation medium alone. Hibernation consisted of cool storage for 6, 9, 12, or 15 days at 4°C in 500 μ L of a low-sodium, phosphate-buffered, calcium-free storage medium.^{1,18,30} For GDNF-supplemented hibernation, recombinant human GDNF (1 μ g/ml; Prepro Tech Inc., Rocky Hill, NJ) was added to the hibernation medium on the 1st day of hibernation.

Cell Culture

Ventral mesencephalon tissue was incubated in 0.1% trypsin in 0.05% DNase/DMEM for 20 minutes at 37°C and then washed in 0.05% DNase/DMEM to stop trypsin activity (trypsin, DNase, and DMEM; Sigma, Chicago, IL). The tissue was mechanically dissociated by trituration using successively smaller sterilized micropipette tips until a "chunky" cell suspension was achieved. The cell suspension was centrifuged at 300 G for 5 minutes by

using a small bench-top centrifuge, and the cell pellet was subsequently resuspended in culture medium. Cell count and viability was determined using the trypan blue exclusion test. Cell suspensions were plated onto a poly-L-lysine-coated substrate (10 μ g/ml; Sigma) at 200,000 cells/cm² in serum-free media consisting of DMEM/F12 (Sigma) 3:1, B27 (Gibco, Burlington, ON, Canada) 1:50 penicillin 100 U/ml, and streptomycin 100 μ g/ml. All cultures were maintained at 37°C in a humidified incubator with a 5% CO₂ atmosphere for 48 hours.

Tyrosine Hydroxylase Immunohistochemistry and Cell Counts

Immunohistochemistry for TH was conducted on 4% paraformaldehyde fixed slides. Briefly, cell cultures were quenched in 3% H₂O₂/10% MeOH for 10 minutes, followed by blocking for 1 hour with normal swine serum in 0.3% Triton-X100. Cell cultures were incubated in primary antibody, rabbit anti-TH (1:750; Pel Freeze, Rogers, AK), overnight at room temperature. They were then incubated in swine anti-rabbit secondary antibody and avidin-biotin complex (Vector Dimension, Burlington, ON, Canada) for 1 hour each. Finally, the cultures were placed in diaminobenzidine/H₂O₂ for 5 to 10 minutes.

Cell counting was performed on cells stained for TH immunoreactivity. Cell counts were made using an eyepiece grid (0.25 $\times$ 0.25 mm divided into a 10 $\times$ 10 grid), counting TH-positive cells and cresyl violet-stained non-TH-positive cells within a fixed area. Sample areas were chosen using a randomized number generator that generated (x and y) coordinates within the total culture area. All data were statistically analyzed using two-way analysis of variance and two-sample t-tests.

RESULTS

All groups yielded healthy-appearing cultures when examined under phase-contrast microscopy during the 48-hour culture period (Fig. 1). The percentage of TH-positive cells was increased in the GDNF-supplemented group for all hibernation periods compared with the groups stored in hibernation medium alone; however, this percentage was only statistically significantly higher in the 6- and 9-day groups (Fig. 2). This increase in percentage of TH immunoreactivity was also significantly higher than fresh (immediately cultured) controls. The percentage of TH-positive cells declined after 9 days in hibernation, becoming significantly less than that observed fresh control values in the 15-day hibernation group.

DISCUSSION

It is estimated that only 1 to 20% of grafted dopaminergic cells survive neural transplantation. Consequently, a large amount of cells—derived from multiple donor fetuses—is required for a transplant to produce meaningful functional outcomes.^{22,28,58} Because of the difficulties surrounding the acquisition of fetal donors and the limited supply of donors of the correct gestational age, tissue storage is desirable to allow collection over several days. Furthermore, complete microbiological screening for infectious diseases in fetal tissues is more easily accom-

Fig. 1. Immunohistochemistry results. Rat fetal VM tissue in culture. Cells were cultured for 48 hours and processed for TH immunoreactivity and double stained with cresyl violet. Fresh control (A), and 6- (B and C), 9- (D and E), 12- (F and G), and 15-day hibernation (H and I), with GDNF-supplemented medium (C, E, G, and I), and control medium (B, D, F, and H). Scale bar = 100 μ m.

plished when storage times are extended. Tissue storage methods such as freeze storage and cool storage for greater than 3 days, however, result in decreased graft volumes and decreased functional recovery in animal studies^{4,11,43,44} and clinical trials.^{41,50} This is thought to be due to increased cell fragility and susceptibility to oxidative stress.^{4,39}

Fortunately, there is evidence that TH-positive cell survival may be augmented by the addition of neuroprotectant factors to the hibernation medium.^{1,15,30,38} Grasbon-Frodl, et al.,¹⁵ have demonstrated increased hibernated rat TH-positive cell survival and functional recovery in the rat model of PD by adding a lazardoid to the hibernation medium. Furthermore, our group has previously demonstrated that addition of the trophic factor GDNF to the

hibernation medium as well as to the cell suspension prior to transplantation increases the absolute number of surviving TH cells per graft, compared with using tissue untreated with GDNF or tissue treated with GDNF only at the cell suspension stage.¹ We subsequently demonstrated that adding GDNF to the hibernation medium resulted in increased fiber density in the zone surrounding the graft, as well as increased functional recovery as measured by the adjusting step test in the rat model of PD.³⁰ We have also observed that human VM tissue stored for 6 days in non-GDNF-supplemented hibernation medium undergoes a 31% decline in TH-positive cell populations compared with fresh controls and tissue hibernated with GDNF.³²

In the present study, we examined GDNF-supplemented hibernation for extended time periods of 6 to 15 days. The GDNF-supplemented hibernation promotes a significant increase of the percentage of TH immunoreactivity cells when tissue is stored up to 9 days at 4°C ($p < 0.02$) compared with both fresh control (immediately cultured) and non-GDNF-supplemented hibernation groups. This increase is not observed at 12 and 15 days ($p > 0.05$). Our results suggest that storing fetal tissue in hibernation media for up to 9 days is beneficial, when supplemented with GDNF. This observation has important practical implications for neural transplantation because hibernation with GDNF will enhance the percentage of dopaminergic cells and allow the pooling of fetal tissue over several days, enabling a sufficient quantity of VM cells to be collected to produce meaningful functional outcomes in transplanted patients undergoing transplantation.

Although the precise molecular mechanism(s) underlying the “dopaminotrophic” effect of GDNF remains unclear, one suggested mechanism is the suppression of caspase activity.⁴⁶ Caspases are mediators of neuronal apoptosis,¹⁷ and reactive oxidative stress has been demonstrated to cause apoptosis via caspase activation.⁵⁷ Hibernation of tissue may lead to the buildup of intracellular free radicals due to ischemia, and reactive oxidative metabolites may be released from lysis of cells.^{5,15} Thus GDNF may prevent oxidative stress-induced apoptosis by preventing caspase activation.

It is likely that the antiapoptotic effect of GDNF involves protein synthesis via induction of nuclear transcription. Sawada and colleagues⁴⁶ have reported that simultaneous administration of GDNF with apoptosis-inducing neurotoxins to midbrain neurons fails to rescue the neurons from the action of the toxins.⁴⁶ This observation is in line with data obtained by our group showing that GDNF-supplemented hibernation of VM tissue prior to transplantation led to significantly increased fiber outgrowth and percentage of TH-positive cells per graft, compared with transplants of fresh VM tissue grafted with (but not stored in) GDNF. These data would appear to suggest that the antiapoptotic actions of GDNF require the de novo synthesis of proteins.⁴⁶

Preincubation with neurotrophic factors may rescue a population of cells that die early in hibernation or in transplanted grafts. Sauer and Brundin⁴³ noted that although there was a significant decrease in TH-positive cell populations of VM hibernated for 24 hours, as determined by cell-smear immunohistochemistry, there was no difference in TH-positive cell populations in grafts of fresh tissue compared with tissue hibernated for 2 days. They pro-

Fig. 2. Graph demonstrating the effect of GDNF supplementation in long-term hibernation on percentage of TH-immunoreactive cell population. * = significant difference from fresh control culture ($p < 0.03$). ** = significant difference from partner hibernation control culture ($p < 0.02$). + = significantly smaller percentage than fresh control culture ($p < 0.0001$).

posed that there exists a population of TH-positive cells destined to die within the first 24 hours in hibernation or after grafting. Preincubation with GDNF in the cell hibernation medium may rescue this particular cell population from early cell death.⁶¹

CONCLUSIONS

Glial cell line-derived neurotrophic factor has been shown to enhance VM grafts and increase cell survival in the rat model of PD, as well as prevent the decline in human fetal VM TH-positive cell survival that is typically seen beyond 3 days in cool storage. In the present study we found that exposing rat VMs to GDNF during tissue hibernation increases the percentage of TH-immunoreactive cell populations for up to 9 days following extraction from fetuses, compared with fresh (immediately cultured) control. This finding bears clinical importance because the technique of GDNF-supplemented hibernation of fetal VM cells will enable longer storage periods of graft material, a critical practical issue in neural transplantation for PD.

References

1. Apostolides C, Sanford E, Hong M, et al: Glial cell line-derived neurotrophic factor improves intrastriatal graft survival of stored dopaminergic cells. **Neuroscience** **83**:363–372, 1998
2. Bjorklund A, Stenevi U: Reconstruction of the nigrostriatal dopamine pathway by intracerebral nigral transplants. **Brain Res** **177**:555–560, 1979
3. Brundin P, Strecker RE, Widner H, et al: Human fetal dopamine neurons grafted in a rat model of Parkinson's disease: immunological aspects, spontaneous and drug-induced behavior, and dopamine release. **Exp Brain Res** **70**:192–208, 1988
4. Collier TJ, Gallagher MJ, Sladek CD: Cryopreservation and storage of embryonic rat mesencephalic dopamine neurons for one year: comparison to fresh tissue in culture and neural grafts. **Brain Res** **623**:249–256, 1993
5. Dong JF, Datta A, Hitchcock ER: Susceptibility of human foetal brain tissue to cool- and freeze-storage. **Brain Res** **621**:242–248, 1993
6. Durbec P, Marcos-Gutierrez CV, Kilkenny C, et al: GDNF signalling through the Ret receptor tyrosine kinase. **Nature** **381**:789–793, 1996
7. Eggert K, Schlegel J, Oertel W, et al: Glial cell line-derived neurotrophic factor protects dopaminergic neurons from 6-hydroxydopamine toxicity in vitro. **Neurosci Lett** **269**:178–182, 1999
8. Freed CR, Greene PE, Breeze RE, et al: Transplantation of embryonic dopamine neurons for severe Parkinson's disease. **N Engl J Med** **344**:710–719, 2001
9. Freed CR, Breeze RE, Leehey MA, et al: Eight years experience with fetal neurotransplantation in patients with advanced Parkinson's Disease. **Soc Neurosci Abst** **22**:1210, 1996
10. Freeman TB, Olanow CW, Hauser RA, et al: Bilateral fetal nigral transplantation into the postcommissural putamen in Parkinson's disease. **Ann Neurol** **38**:379–388, 1995
11. Frodl EM, Duan WM, Sauer H, et al: Human embryonic dopamine neurons xenografted to the rat: effects of cryopreservation and varying regional source of donor cells on transplant survival, morphology and function. **Brain Res** **647**:286–298, 1994
12. Gage FH, Brundin P, Isacson O, et al: Rat fetal brain tissue grafts survive and innervate host brain following five day pre-graft tissue storage. **Neurosci Lett** **60**:133–137, 1985

13. Gage NH, Dunnett SB, Stenevi U, et al: Aged rats: recovery of motor impairments by intrastriatal nigral grafts. **Science** **221**: 966–969, 1983
14. Granholm AC, Mott JL, Bowenkamp K, et al: Glial cell line-derived neurotrophic factor improves survival of ventral mesencephalic grafts to the 6-hydroxydopamine lesioned striatum. **Exp Brain Res** **116**:29–38, 1997
15. Grashon-Frodil EM, Nakao N, Brundin P: The lazaroïd U-83836E improves the survival of rat embryonic mesencephalic tissue stored at 4 degrees C and subsequently used for cultures or intracerebral transplantation. **Brain Res Bull** **39**:341–347, 1996
16. Jing S, Wen D, Yu Y, et al: GDNF-induced activation of the ret protein tyrosine kinase is mediated by GDNFR- α , a novel receptor for GDNF. **Cell** **85**:1113–1124, 1996
17. Juin P, Pelletier M, Oliver L, et al: Induction of a caspase-3-like activity by calcium in normal cytosolic extracts triggers nuclear apoptosis in a cell-free system. **J Biol Chem** **273**: 17559–17564, 1998
18. Kawamoto JC, Barrett JN: Cryopreservation of primary neurons for tissue culture. **Brain Res** **384**:84–93, 1986
19. Kearns CM, Gash DM: GDNF protects nigral dopamine neurons against 6-hydroxydopamine in vivo. **Brain Res** **672**: 104–111, 1995
20. Kopyov OV, Jacques D, Lieberman A, et al: Clinical study of fetal mesencephalic intracerebral transplants for the treatment of Parkinson's disease. **Cell Transplant** **5**:327–337, 1996
21. Kopyov OV, Jacques DS, Lieberman A, et al: Outcome following intrastriatal fetal mesencephalic grafts for Parkinson's patients is directly related to the volume of grafted tissue. **Exp Neurol** **146**:536–545, 1997
22. Kordower JH, Freeman TB, Snow BJ, et al: Neuropathological evidence of graft survival and striatal reinnervation after the transplantation of fetal mesencephalic tissue in a patient with Parkinson's disease. **N Engl J Med** **332**:1118–1124, 1995
23. Kordower JH, Rosenstein JM, Collier TJ, et al: Functional fetal nigral grafts in a patient with Parkinson's disease: chemoanatomic, ultrastructural, and metabolic studies. **J Comp Neurol** **370**:203–230, 1996
24. Kriegstein K, Suter-Crazzolara C, Fischer WH, et al: TGF- β superfamily members promote survival of midbrain dopaminergic neurons and protect them against MPP⁺ toxicity. **Embo J** **14**:736–742, 1995
25. Kriegstein K, Suter-Crazzolara C, Unsicker K: Development of mesencephalic dopaminergic neurons and the transforming growth factor- β superfamily. **J Neural Transm Suppl** **46**: 209–216, 1995
26. Levivier M, Dethy S, Rodesch F, et al: Intracerebral transplantation of fetal ventral mesencephalon for patients with advanced Parkinson's disease. Methodology and 6-month to 1-year follow-up in 3 patients. **Stereotact Funct Neurosurg** **69**: 99–111, 1997
27. Lin LF, Doherty DH, Lile JD, et al: GDNF: a glial cell line-derived neurotrophic factor for midbrain dopaminergic neurons. **Science** **260**:1130–1132, 1993
28. Lindvall O, Brundin P, Widner H, et al: Grafts of fetal dopamine neurons survive and improve motor function in Parkinson's disease. **Science** **247**:574–577, 1990
29. Lopez-Lozano JJ, Bravo G, Brera B, et al: Long-term improvement in patients with severe Parkinson's disease after implantation of fetal ventral mesencephalic tissue in a cavity of the caudate nucleus: 5-year follow up in 10 patients. Clinica Puerta de Hierro Neural Transplantation Group. **J Neurosurg** **86**: 931–942, 1997
30. Mehta V, Hong M, Spears J, et al: Enhancement of graft survival and sensorimotor behavioral recovery in rats undergoing transplantation with dopaminergic cells exposed to glial cell line-derived neurotrophic factor. **J Neurosurg** **88**:1088–1095, 1998
31. Mehta V, Spears J, Mendez I: Neural transplantation in Parkinson's disease. **Can J Neurol Sci** **24**:292–301, 1997
32. Mendez I, Dagher A, Hong M, et al: Enhancement of survival of stored dopaminergic cells and promotion of graft survival by exposure of human fetal nigral tissue to glial cell line-derived neurotrophic factor in patients with Parkinson's disease. Report of two cases and technical considerations. **J Neurosurg** **92**: 863–869, 2000
33. Mendez I, Dagher A, Hong M, et al: Simultaneous intrastriatal and intranigral fetal dopaminergic grafts in patients with Parkinson disease: a pilot study. Report of three cases. **J Neurosurg** **96**:589–596, 2002
34. Mendez I, Elisevich K, Flumerfelt B: Dopaminergic innervation of substance P-containing striatal neurons by fetal nigral grafts: an ultrastructural double-labeling immunocytochemical study. **J Comp Neurol** **308**:66–78, 1991
35. Mendez I, Elisevich K, Flumerfelt BA: GABAergic synaptic interactions in the substantia nigra. **Brain Res** **617**:274–284, 1993
36. Mendez I, Elisevich K, Naus C, et al: Restoration of nigrostriatal synaptic circuitry, striatal mRNA expression, and motor symmetry following embryonic substantia nigra grafts. **Clin Neurosurg** **38**:180–209, 1992
37. Mendez I, Sadi D, Hong M: Reconstruction of the nigrostriatal pathway by simultaneous intrastriatal and intranigral dopaminergic transplants. **J Neurosci** **16**:7216–7227, 1996
38. Nakao N, Frodl EM, Duan WM, et al: Lazaroids improve the survival of grafted rat embryonic dopamine neurons. **Proc Natl Acad Sci USA** **91**:12408–12412, 1994
39. Nikkiah G, Eberhard J, Olsson M, et al: Preservation of fetal ventral mesencephalic cells by cool storage: in-vitro viability and TH-positive neuron survival after microtransplantation to the striatum. **Brain Res** **687**:22–34, 1995
40. Potter ED, Ling ZD, Carvey PM: Cytokine-induced conversion of mesencephalic-derived progenitor cells into dopamine neurons. **Cell Tissue Res** **296**:235–246, 1999
41. Redmond DE Jr, Leraneth C, Spencer DD, et al: Fetal neural graft survival. **Lancet** **336**:820–822, 1990
42. Redmond DE Jr, Naftolin F, Collier TJ, et al: Cryopreservation, culture, and transplantation of human fetal mesencephalic tissue into monkeys. **Science** **242**:768–771, 1988
43. Sauer H, Brundin P: Effects of cool storage on survival and function of intrastriatal ventral mesencephalon grafts. **Restor Neurol Neurosci** **2**:123–135, 1991
44. Sauer H, Frodl EM, Kupsch A, et al: Cryopreservation, survival and function of intrastriatal fetal mesencephalic grafts in a rat model of Parkinson's disease. **Exp Brain Res** **90**:54–62, 1992
45. Sauer H, Rosenblad C, Bjorklund A: Glial cell line-derived neurotrophic factor but not transforming growth factor β 3 prevents delayed degeneration of nigral dopaminergic neurons following striatal 6-hydroxydopamine lesion. **Proc Natl Acad Sci USA** **92**:8935–8939, 1995
46. Sawada H, Ibi M, Kihara T, et al: Neuroprotective mechanism of glial cell line-derived neurotrophic factor in mesencephalic neurons. **J Neurochem** **74**:1175–1184, 2000
47. Shults CW, Kimber T, Martin D: Intrastriatal injection of GDNF attenuates the effects of 6-hydroxydopamine. **Neuroreport** **7**:627–631, 1996
48. Sinclair SR, Svendsen CN, Torres EM, et al: GDNF enhances dopaminergic cell survival and fiber outgrowth in embryonic nigral grafts. **Neuroreport** **7**:2547–2552, 1996
49. Sladek JR Jr, Redmond DE Jr, Collier TJ, et al: Fetal dopamine neural grafts: extended reversal of methylphenyltetrahydropyridine-induced parkinsonism in monkeys. **Prog Brain Res** **78**: 497–506, 1988
50. Spencer DD, Robbins RJ, Naftolin F, et al: Unilateral transplantation of human fetal mesencephalic tissue into the caudate nucleus of patients with Parkinson's disease. **N Engl J Med** **327**:1541–1548, 1992

51. Stromberg I, Bjorklund L, Johansson M, et al: Glial cell line-derived neurotrophic factor is expressed in the developing but not adult striatum and stimulates developing dopamine neurons in vivo. **Exp Neurol** **124**:401–412, 1993
52. Tang FI, Tien LT, Zhou FC, et al: Intranigral ventral mesencephalic grafts and nigrostriatal injections of glial cell line-derived neurotrophic factor restore dopamine release in the striatum of 6-hydroxydopamine-lesioned rats. **Exp Brain Res** **119**:287–296, 1998
53. Tomac A, Lindqvist E, Lin LF, et al: Protection and repair of the nigrostriatal dopaminergic system by GDNF in vivo. **Nature** **373**:335–339, 1995
54. Treanor JJ, Goodman L, de Sauvage F, et al: Characterization of a multicomponent receptor for GDNF. **Nature** **382**:80–83, 1996
55. Trupp M, Arenas E, Fainzilber M, et al: Functional receptor for GDNF encoded by the c-ret proto-oncogene. **Nature** **381**:785–789, 1996
56. Trupp M, Belluardo N, Funakoshi H, et al: Complementary and overlapping expression of glial cell line-derived neurotrophic factor (GDNF), c-ret proto-oncogene, and GDNF receptor- α indicates multiple mechanisms of trophic actions in the adult rat CNS. **J Neurosci** **17**:3554–3567, 1997
57. Turner NA, Xia F, Azhar G, et al: Oxidative stress induces DNA fragmentation and caspase activation via the c-Jun NH₂-terminal kinase pathway in H9c2 cardiac muscle cells. **J Mol Cell Cardiol** **30**:1789–1801, 1998
58. Widner H, Tetrad J, Rehncrona S, et al: Bilateral fetal mesencephalic grafting in two patients with parkinsonism induced by 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP). **N Engl J Med** **327**:1556–1563, 1992
59. Winkler C, Sauer H, Lee CS, et al: Short-term GDNF treatment provides long-term rescue of lesioned nigral dopaminergic neurons in a rat model of Parkinson's disease. **J Neurosci** **16**:7206–7215, 1996
60. Yurek DM: Glial cell line-derived neurotrophic factor improves survival of dopaminergic neurons in transplants of fetal ventral mesencephalic tissue. **Exp Neurol** **153**:195–202, 1998
61. Zawada WM, Zastrow DJ, Clarkson ED, et al: Growth factors improve immediate survival of embryonic dopamine neurons after transplantation into rats. **Brain Res** **786**:96–103, 1998

Manuscript received September 16, 2002.

Accepted in final form October 16, 2002.

Address reprint requests to: Ivar Mendez, M.D., Ph.D., Neural Transplantation Laboratory, Department of Anatomy and Neurobiology, Sir Charles Tupper Medical Building, Dalhousie University, Halifax, Nova Scotia, B3H 4H7, Canada. email: mendez@is.dal.ca.